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Review article

Single-cell and spatial transcriptomics: Advances in heart development and disease applications

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ABSTRACT

Current transcriptomics technologies, including bulk RNA-seq, single-cell RNA sequencing (scRNA-seq), single-nucleus RNA-sequencing (snRNA-seq), and spatial transcriptomics (ST), provide novel insights into the spatial and temporal dynamics of gene expression during cardiac development and disease processes. Cardiac development is a highly sophisticated process involving the regulation of numerous key genes and signaling pathways at specific anatomical sites and developmental stages. Exploring the cell biological mechanisms involved in cardiogenesis also contributes to congenital heart disease research. Meanwhile, the severity of distinct heart diseases, such as coronary heart disease, valvular disease, cardiomyopathy, and heart failure, is associated with cellular transcriptional heterogeneity and phenotypic alteration. Integrating transcriptomic technologies in the clinical diagnosis and treatment of heart diseases will aid in advancing precision medicine. In this review, we summarize applications of scRNA-seq and ST in the cardiac field, including organogenesis and clinical diseases, and provide insights into the promise of single-cell and spatial transcriptomics in translational research and precision medicine.

Contents

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1. Introduction

The heart is essential for maintaining circulation in the human body [1]. Cardiovascular disease is the leading cause of death and diminished life expectancy in humans, and the cases and deaths of cardiovascular disease have continued to increase worldwide over the past 30 years [2]. In recent years, with the rapid advancement of genome sequencing and big data technology, clinical diagnosis and treatment patterns are also gradually changing from traditional medicine to precision medicine [3,4]. The concept of precision medicine was first introduced by the National Research Council in 2011, aiming to elevate the understanding of disease from the clinical symptom level to the cellular and molecular level [5]. Thus, continuing in-depth research on microscopic aspects of the cellular and molecular mechanisms of cardiogenesis and cardiology is important for advancing precision medicine treatment of cardiovascular diseases [6–9].

From the biological perspective, the cell is the basic unit that constitutes the life activities of mammals [10,11]. Multiple cell types and developmental stages are often present within the same tissue or organ [12,13]. While the spatiotemporal gene expression features largely determine cell fate and function in the internal environment [14].

Single-cell RNA sequencing (scRNA-seq) technology has been widely applied in recent years. This technology enables analysis of intracellular gene expression at the individual cell level, and construction and comparison of the cellular landscapes of tissues and organs under physiological and disease conditions, provided that the number of cells is sufficient [15–18] (Fig. 1). As a result, scRNA-seq can be used to explore dynamic changes in cellular gene expression in tissues or organs during development or disease progression, thereby providing new insights into life science and clinical diagnosis and treatment [19]. One challenge of this technology is that cells lose their original spatial information and in situ intercellular communication networks through the action of digestive enzymes during tissue dissociation [20,21]. Spatial transcriptomics (ST) technologies address this challenge by integrating gene expression and cell or tissue localization information to visualize the RNA molecular profiles within a defined tissue region [12,22,23] (Fig. 1). In the past few years, ST technologies has been used to analyze cardiovascular samples in healthy and diseased states (Table 1). When applied to organ development and disease, ST technologies can provide spatially resolved transcriptional information on tissues [24,25]. Hence, the integration of scRNA-seq and ST can enable the assessment of cellular gene expression in multiple dimensions of time, space, species, and disease, and support spatiotemporal transcriptomic studies on organs at the single-cell level [26–28]. Besides, when applied to clinical medicine, these methods can help identify therapeutic targets for cardiovascular diseases and facilitate precision medicine for complicated cases (Fig. 2).

Thus, in this review, we summarized the findings of scRNA-seq and ST in heart development and diseases, and elaborated on the significance of the above transcriptomics techniques in precision medicine for heart disease. Furthermore, the limitations of scRNA-seq and ST technology and several viewpoints on the future outlook are listed at the end.

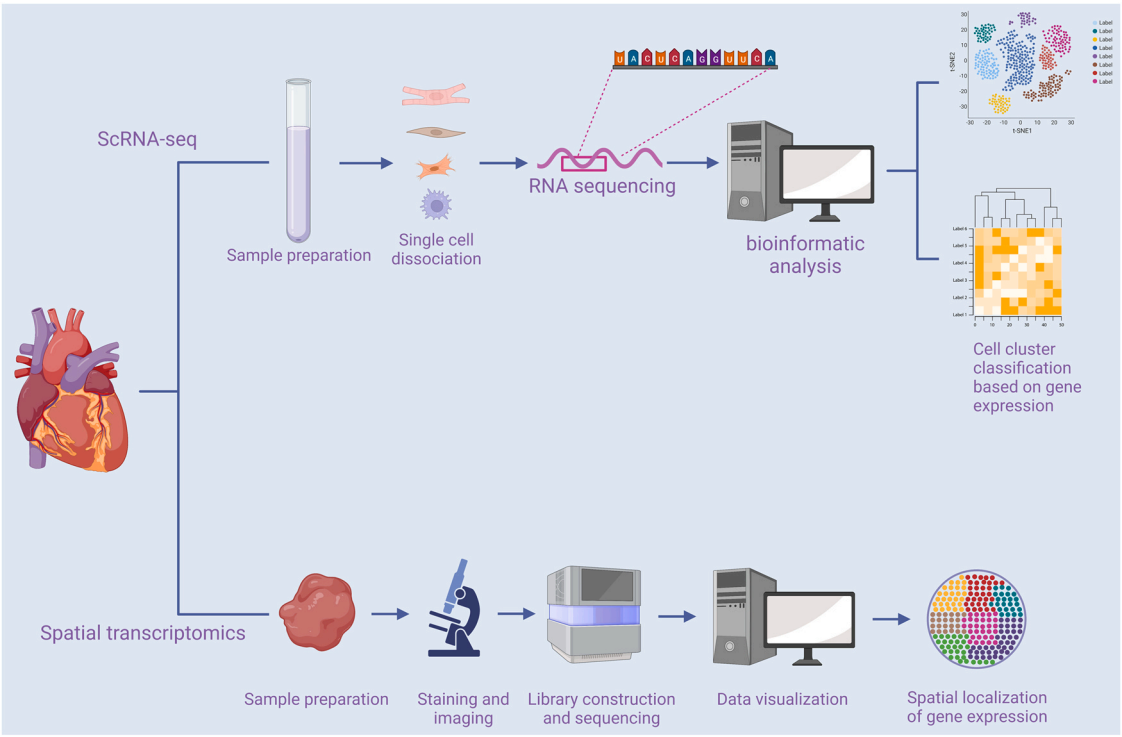

Fig. 1. Workflow of scRNA-seq and ST technologies.

2. Applications of scRNA-seq and ST in heart development

The heart is the first functional organ arising during human embryonic development, and it supports blood circulation in the third week after fertilization [29,30]. Heart development is a precise and complex process and is regulated by multiple genes and signaling pathways [31,32]. During cardiogenesis, cells that constitute different anatomical structures (e.g., ventricle, atrium, and outflow tract (OFT)) may have distinct origins and cell fates, which is closely associated with the spatiotemporal heterogeneity of gene expression patterns [33]. Currently, single-cell and spatial transcriptomics can provide a more comprehensive cell atlas and contribute to a deeper understanding of the cell biology processes during organogenesis [34–37]. In this part, we summarized the major findings of scRNA-seq and ST applied in heart development from the organ, anatomical structure, cell, and gene perspectives.

2.1. Transcriptional landscape of the developing heart

2.1.1. Human heart

Asp et al. characterized the spatiotemporal expression of cardiac genes and investigated the roles of several cell populations in different stages of human heart development. They observed differences in gene expression patterns among heart regions from 4.5 to 9 weeks post-conception (PCW), such as conservation of gene expression in ventricular muscle, atrial muscle, and OFT cells. Gene expression shows spatiotemporal dynamic changes among valvular, vascular, and annulus fibrosus cells. Beyond normal atrial and ventricular cardiomyocytes, a new cardiomyocyte type, Myoz2-enriched cardiomyocytes, with specific expression of the Myoz2 and Fabp3 genes, has been identified. This cell subpopulation exists in both atrial and ventricular regions. Of note, cardiac neural crest cells (CNCCs) and Schwann progenitor cells show temporal specificity

Table 1
Spatial transcriptomics studies of the cardiovascular system.

Species	Developmental stage	Sample	Disease	Technology	Ref.
Human	Embryonic (4.5–5, 6.5 and 9 PCW)[a]	Heart	NA[b]	"Spatial transcriptomics" devised by Ståhl et al.	[24,34]
Human	Adult	Aortic Tissue	Stanford type A aortic dissection	10x Visium	[141]
Human	Adult	Heart	ACM[c]	Tomo-Seq	[120]
Human	Adult	Heart	Myocardial infarction	10x Visium	[106]
Mouse	Adult	Heart	Ischemia-reperfusion injury	Tomo-Seq	[92]
Mouse	Postnatal (P3, P7, P14 and P21)[d]	Heart	Apical resection surgery	10x Visium	[43]
Mouse	Embryonic (E13.5 and E15.5)[e]	Heart	Prdm16 knockout	10x Visium	[73]
Mouse	Adult	Heart	Myocardial infarction	10x Visium	[107]
Mouse	Adult	Heart	Myocardial infarction	10x Visium	[126]
Mouse	Adult	Heart	Myocardial infarction	10x Visium	[93]
Mouse	Adult	Heart	Viral myocarditis	10x Visium and Slide-Seq	[123]
Mouse	Adult	Heart	After base-targeted irradiation	10x Visium	[146]
Chicken	Embryonic (E4, E7, E10 and E14)	Heart	NA	10x Visium	[45]

[a] PCW: post-conception weeks
[b] NA: not applicable
[c] ACM: arrhythmogenic cardiomyopathy
[d] P: postnatal day
[e] E: embryonic day

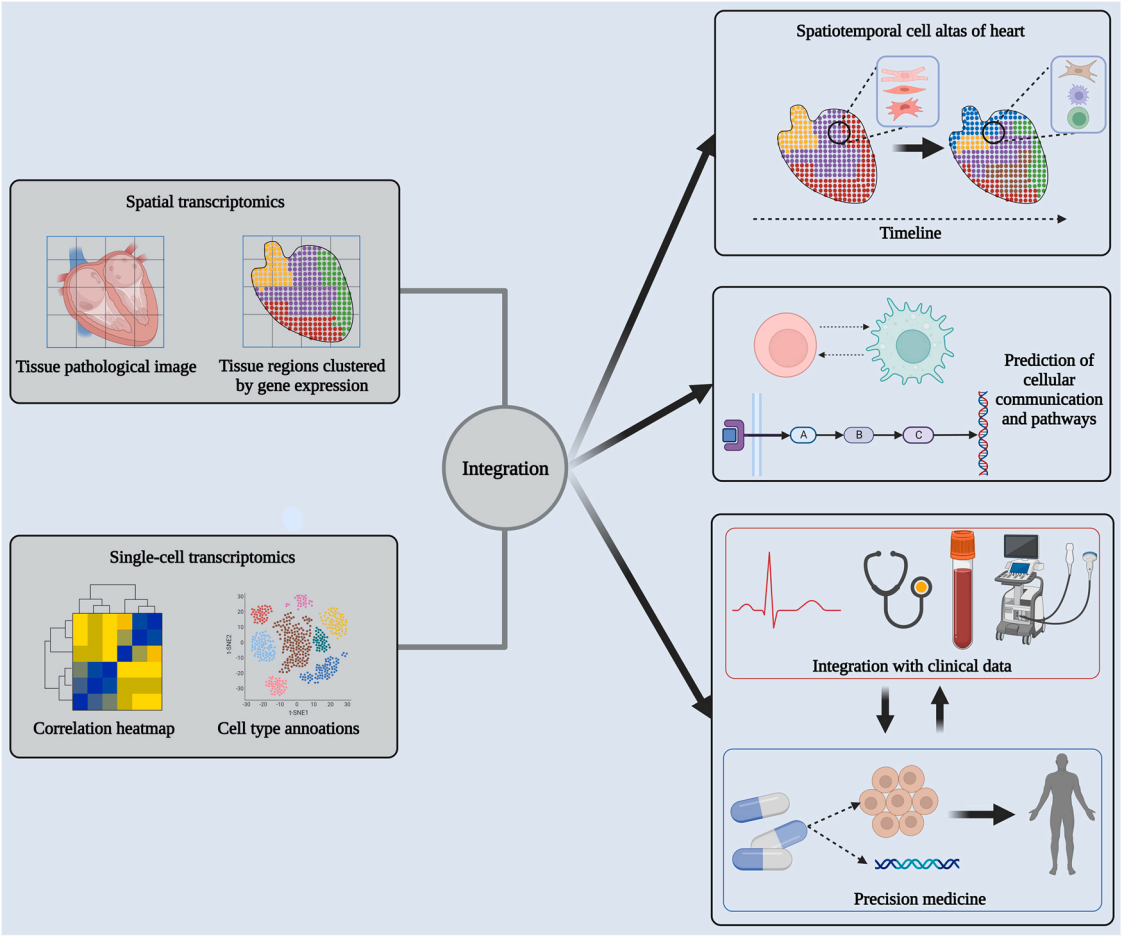

Fig. 2. Integration of single-cell and spatial transcriptomics in the cardiovascular field.

during cardiac OFT development: CNCCs appear mainly at 4.5–6.5 PCW, and Schwann progenitor cells appear mainly at 6.5–9 PCW. CNCCs have been found to participate in OFT septation and reconstruction, and are associated with human congenital heart defects [34,38,39].

Similarly, Cui et al. analyzed the transcriptional map of 4000 human heart cells from 5 to 25 weeks of pregnancy by scRNA-seq and identified four main cell populations: cardiomyocytes, cardiac fibroblasts, endothelial cells (ECs), and valvar interstitial cells. Atrial and ventricular cardiomyocytes show different transcriptional characteristics in the early stages of development, and the gene expression of cardiomyocytes and fibroblasts changes dynamically during development. For example, extracellular matrix (ECM) gene expression is rapidly upregulated in cardiomyocytes and fibroblasts from week 5 to week 6. The NOTCH and BMP signaling pathways play synergistic roles in the differentiation and maturation of cardiomyocytes, whereas valvar interstitial cells are involved in the remodeling process of myocardial trabeculae from week 5 to week 8. Moreover, a group of human-specific cardiac cell markers was identified through comparison with mouse heart scRNA-seq datasets produced by Li et al. and Xiao et al. [40–42].

2.1.2. Mouse heart

On the basis of scRNA-seq data for mouse heart at embryonic day (E) 10.5, Li et al. found that transcriptional profiles change with cell cycle state, thereby affecting cardiogenesis. Cardiomyocytes in the G2 and M phases show down-regulation of the sarcomere and cytoskeleton marker gene expression. Cell location-specific signaling molecules have also been identified and may influence the proliferation of the nearby cell types. Interestingly, the pro-mitotic Wnt signaling ligand is specifically expressed in the epicardium. In contrast, the anti-mitotic Tgfb1 signaling ligand is specifically expressed in the endocardium and may be involved in the development of trabecular cardiomyocytes [41].

On the basis of bulk RNA-sequencing and ST data for mouse hearts at postnatal day (P) 0, Misra et al. observed dynamic spatial changes in gene expression profiles during heart maturation. Epicardial and endocardial cell clusters were defined on the basis of ST data. Similar to the developing human heart, ST analysis indicated that the transcriptional profiles differ between the ventricle and atrium [40]. For example, the Myl2 gene is enriched in the ventricle, whereas Nppa, Fgf12, and Sln are highly expressed in the atrium. Significant gene expression differences have also been found between the left and right atrium [43].

2.1.3. Chicken heart

Previous single-cell analyses have shown the widespread presence of epithelial-mesenchymal transition (EMT) in organogenesis [44]. By combining the scRNA-seq and ST data of embryonic chicken hearts from E4 to E14, Mantri et al. constructed a transcriptome atlas with a single cell and spatial resolution. Transcriptional profile differences were observed between epithelial and mesenchymal cells. ECM-associated genes, such as AGRN, EGFL7, POSTN, and FN, were found to be involved in the EMT process of epicardial progenitor-derived cells. In addition, persistent enrichment in thymosin beta-4 has been observed during coronary vasculature development [45].

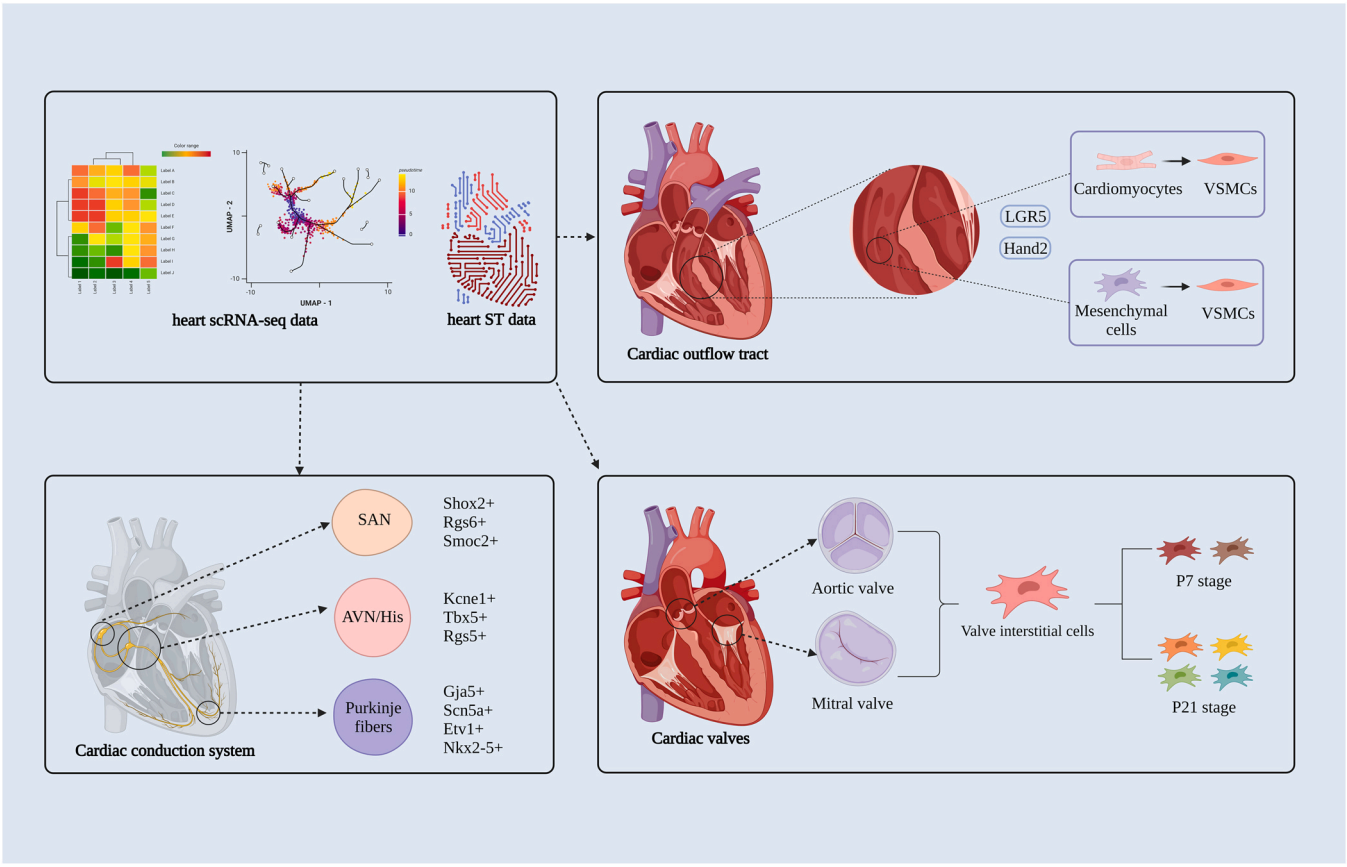

Fig. 3. Single-cell characterization of the cardiac outflow tract, valves, and conduction system.

2.2. Key anatomical structures of the heart

In this section, we summarized the results of several scRNA-seq studies on cardiac anatomical structures.

2.2.1. Cardiac outflow tract

During embryonic cardiogenesis, the cardiac OFT can be reconstructed into the main artery and pulmonary artery and is associated with the morphogenesis of the semilunar valves and the formation of the coronary artery. Abnormal development of the cardiac OFT structure causes many congenital heart diseases [46]. Liu et al. performed scRNA-seq on 55,611 OFT cells in the early, middle, and late stages of cardiac septation. According to clustering analysis, six cardiac cell types participate in OFT development. Convergent development of the vascular smooth muscle cell (VSMC) lineage has been found to occur during OFT development. For example, cell subpopulations involved in both the trans-differentiation of cardiomyocytes to VSMCs and the differentiation of mesenchymal cells to VSMCs have been observed [47]. Moreover, LGR5^{+} cono-ventricular cardiac progenitor cells (CPCs) were indicated to specifically express in the proximal OFT of the human heart at embryonic week 4 to week 5 [48] (Fig. 3).

2.2.2. Cardiac valves

Cardiac valves play an important role in the circulation of the heart by ensuring that blood flows in the correct direction [49]. Hulin's scRNA-seq of P7 and P30 mouse heart aortic and mitral valve cells revealed the existence of different cell subpopulation compositions at both sites and the dynamic changes in the gene transcriptional profiles of interstitial valve cells. P7 phase valve interstitial cells consisted primarily of two different interstitial cell subpopulations expressing collagen and glycosaminoglycan, whereas P30 stage valve interstitial cells included four subpopulations differentially expressing complement factors, ECM proteins, and osteogenic genes [50] (Fig. 3).

2.2.3. Cardiac conduction system

The cardiac conduction system (CCS) generates and conducts electrical impulses and maintains the normal rhythm of the heartbeat [51]. Goodyer's scRNA-seq of the CCS on the embryonic day 15, the first transcriptional profiling assessment of the CCS at single-cell resolution, revealed specific genes for various parts of the cardiac conduction system (including the sinoatrial node (SAN), atrioventricular node (AVN), the bundle of His (His), and Purkinje fibers). The genes, including HCN4, Gjc1, Ntm, Igfbp5, Cpne5, and Cacna2d2, were specifically expressed in CCS. In addition, the Shox2, Rgs6, and Smoc2 genes are specifically expressed in SAN; Kcne1, Tbx5, and Rgs5 are specifically expressed in AVN and His; Gja5, Scn5a, Etv1, Nkx2–5 were specifically expressed in Purkinje fibers. Research has indicated potential markers for studies to improve diagnosis, treatment, and interventions for arrhythmias and heart block [52] (Fig. 3). In CCS, the SAN is the origin of electrical activity. Previous snRNA-seq of SAN indicated specificity in metabolism, ion channels, and ECM gene expression, and suggested that the Vsnl1 gene may be a potential marker of SAN core cell clusters in mice [53,54].

2.2.4. Intrinsic cardiac nervous system

The intrinsic cardiac nervous system participates in cardiac electrophysiological activity by modulating CCS [55]. Moss et al. constructed a cellular transcriptomic atlas of the intrinsic cardiac nervous system in the porcine heart by scRNA-seq. Cholinergic and catecholaminergic genes are co-expressed in the right atrial ganglionic plexus, thus suggesting that the right atrial ganglionic plexus

has multiple potential neuronal phenotypes and plays a key role in regulating critical mediators of SAN activity [56].

2.3. Cardiac cell biological activities

During cardiogenesis, CNCCs were found to differentiate into pericytes and microvascular smooth muscle cells (mVSMCs). Through a single-cell transcriptional landscape construction of mouse CNCCs from E10.5 to P7, the transition from CNCC-derived pericytes to microvascular smooth muscle cells (mVSMCs) and the potential regulators of CNCC differentiation have been identified. For example, the Egr genes and Hox genes are thought to be involved in the process [57].

With heart maturation, cardiomyocytes and fibroblasts become the fundamental components of heart function [11,58]. Cardiomyocytes in the proliferative area in zebrafish show transcriptional characteristics of metabolic reprogramming, including a decrease in mitochondrial gene expression and an increase in glycolytic gene expression. Furthermore, Nrg1/ErbB2 signaling participates in regulating glycolysis and cell proliferation in zebrafish cardiomyocytes [59]. As for cardiac fibroblasts, RNA velocity was defined as the point of transformation of cardiac fibroblasts to cardiomyocyte fate, and the immune response may affect the process by regulating DNA methylation [60]. Besides, an integrated scRNA-seq analysis from postnatal mouse hearts showed that cardiac fibroblasts are key components in the microenvironment in promoting cardiomyocyte maturation. While highly conserved signaling pathways, including CXCR4 and CXCL12-mediated chemotaxis and STAT3 phosphorylation-mediated synthesis of ECM, were found to delay cardiomyocyte maturation after drug targeting [61]. In the meantime, the Hippo kinases Lats1/2 were demonstrated to promote epicardial-fibroblast transition and were associated with ECM composition and coronary vessel formation, according to scRNA-seq of mouse hearts at E13.5 and E14.5 [42].

2.4. Key transcription factors and genes

In this section, we summarized several key transcription factors and genes discovered or validated by scRNA-seq and ST, which are essential for many important cell life processes during cardiac development, such as cell differentiation, proliferation, and migration.

2.4.1. Nkx2-5

Previous studies have suggested that mutations in the Nkx2–5 transcription factors are associated with multiple classes of congenital heart disease [62]. By scRNA-seq, DeLaughter et al. demonstrated lineage-specific maturation defects in the hearts of the Nkx2–5 mutated mice. Nkx2–5 was necessary for cardiomyocyte maturation and the establishment of ventricular structure [63]. Similarly, through scRNA-seq of 2233 mouse embryonic heart cells, Li et al. observed an absence of ventricular transcriptional profiles in Nkx2–5 gene-deficient embryonic mouse cardiomyocytes [64]. Of note, the cell fate transition of CPCs is associated with the open chromatin state influenced by Isl1 and Nkx2–5 [65]. Besides, Nkx2–5 was found to directly bind the Cxcr2 and Cxcr4 genomic loci and activate their transcription in the second heart field, thereby participating in the migration and differentiation of cardiac progenitor cells [66].

2.4.2. BNC1

Gambardell et al. performed scRNA-seq on human pluripotent stem cell-derived epicardial cells and found that the epicardial-specific transcription factors Tcf21 and Wt1 are regulated by transcription factor BNC1. These transcription factors are closely associated with human pluripotent stem cell-derived epicardial cell functional heterogeneity [67].

2.4.3. NR2F2 and HEY2

Churko et al. performed scRNA-seq and bulk RNA-seq on human induced pluripotent stem cell-derived cardiomyocytes at different developmental stages and constructed a transcription factor co-regulatory network among NR2F2, HEY2 and Tbx5. The transcription factors NR2F2 and HEY2 regulated the expression of the atrial-like gene and ventricular-like gene expression profiles, respectively [68].

2.4.4. Hand2

Hand2 has been found to be a specific transcription factor for OFT cells (Fig. 3). In a Hand2-null mouse embryonic model, OFT cardiomyocytes lost their specification properties, and the differentiation and migration of right ventricular cardiomyocytes were also affected [69]. Through scRNA-seq of lncRNA Hand2os1 knockout mouse heart cells, Han et al. observed a chaotic and defective heart gene regulation program in heart development. While Hand2os1 is believed to inhibit cardiomyocyte proliferation by inhibiting the expression of the Hand2 gene [70].

2.4.5. Mesp1

Mesp1 was critical in regulating the expression of several key genes during heart development, including the EMT genes Snai1 and Zeb2, the migration gene Rasgrp3, and the cardiac cell commitment genes Etv2, Hand1, Myl7, Gata4, Flk1, and Pdgfra [71]. In addition, on the basis of scRNA-seq data of embryonic heart cells, the human-specific transcriptional interaction network among Mesp1, LGR5, and ISL1 has been found to potentially promote the development of cono-ventricular cells [48].

2.4.6. HOPX

By scRNA-seq of human pluripotent stem cells (hPSCs), the heart development and hypertrophy regulator HOPX was found to be irreplaceable in hPSC differentiation and hPSC-derived cardiomyocyte maturation [72].

2.4.7. Prdm16

Wu et al. performed scRNA-seq and chromatin immunoprecipitation deep sequencing on Prdm16 cardiomyocyte-specific knockout mouse heart and identified Pdrm16 as an important transcription factor in myocardial densification. Moreover, through ST sequencing, the transcriptional regulation of Prdm16 has been found to be chamber-specific and to be associated with the enrichment of Tbx5 and Hand1 in the left ventricular region [73]. A brief summary of these findings is shown in Fig. 4.

3. Applications of scRNA-seq and ST in cardiovascular diseases

Cardiovascular diseases, primarily including atherosclerosis, hypertension, myocardial infarction (MI), cardiomyopathy, and valvular disease, show commonalities and differences in their pathological mechanisms and gene expression patterns [74–78]. In addition, specific genetic information features and cellular phenotypes exist in different stages of the same disease [79]. Thus, single-cell and spatial transcriptomics are important research methods in cardiology because of their ability to reveal specific cell subpopulations, spatiotemporal specificity of key signaling pathways, and in situ cell-to-cell interactions [80,81]. In this part, we provided an overview of scRNA-seq and ST research findings in various cardiovascular diseases and pathological alterations.

3.1. Atherosclerosis

Atherosclerosis is an important cause of acute cardiovascular events, whose pathological process consists primarily of lipid accumulation and chronic inflammation caused by immune cell infiltration [82]. In single-cell analysis, the atherosclerotic process has

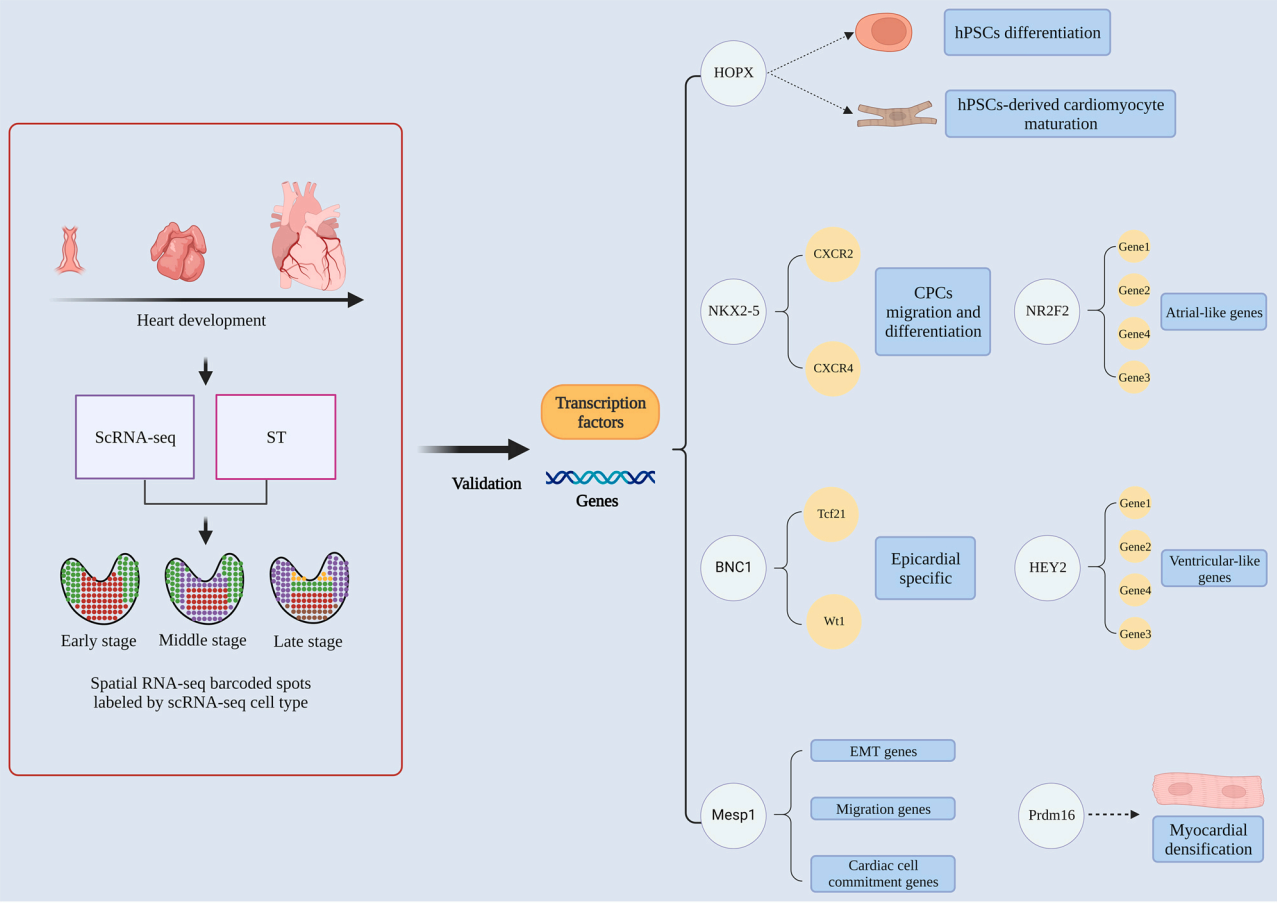

Fig. 4. Key transcription factors and genes in heart development.

been found to involve the transformation of cell types and changes in the expression of specific genes [83].

3.1.1. Smooth muscle cells (SMCs)

Wirka et al. performed scRNA-seq on human and mouse arterial tissues with atherosclerosis and found that SMCs can be converted into fibroblasts through phenotypic regulation, and the gene Tcf21 is involved in atherosclerotic progression by regulating this process [84]. Similarly, Pan et al. found that SMCs can convert into intermediate stem cells, ECs, and monocytes (collectively called "SEM" cells) during atherosclerosis, which in turn can differentiate into macrophage-like and fibrocartilage-like cells. SEM cells have also been found in a public database of human arteriosclerosis. Besides, all-trans retinoic acid has been found to regulate the transformation of SMCs to SEM cells by activating the retinoic acid signaling pathway, thus hindering the progression of arteriosclerosis and promoting the stability of the fibrous cap [85]. At the same time, the transition from SMCs to fibromyocytes has been observed through single-nucleus assay for transposase-accessible chromatin using sequencing (ATAC-seq) of human atherosclerotic lesions [86].

3.1.2. ECs

According to the scRNA-seq data of human atherosclerotic plaques, ECs were divided into four subpopulations: E.0, E.1, E.2, and E.3. The E.3 subgroup expresses the typical SMC markers (ACTA2, NOTCH3, and MYH11), and endothelial to mesenchymal transition (EndMT) may occur in atherosclerotic plaques [87].

3.1.3. Immune cells

The immune cell composition in human and mouse atherosclerotic plaques is similar, and the macrophages and dendritic cells show similar transcriptional profiles [76,88]. Of note, macrophages in an atherosclerosis model were classified into three subsets: resident-like macrophages, TREM2hi macrophages, and inflammatory macrophages. Interestingly, TREM2hi macrophage gene expression is enriched in lipid metabolism and catabolism and also shows osteoclastic characteristics [76]. Meanwhile, by single-cell multi-omics technology, Fernandez et al. constructed a transcriptional profile of immune cells in human atherosclerotic plaques, in which some T cell subsets express T cell exhaustion markers, and macrophages are characterized by alternating activation. A group of T cells expressing PD-1 was also identified [89].

In clinical research, by combining with clinical cohort data, the proportion of specific granulocyte subpopulations in carotid plaques was thought to be predictive of cardiovascular disease events [83]. Similarly, thirty-seven genes associated with the risk of coronary artery disease were identified according to the scRNA-seq on immune cells derived from human atherosclerotic plaques, among which GPX1 and C4orf3 expression is enriched in macrophages, thus providing potential targets for therapeutic drug development [90]. A brief summary of these findings is shown in Fig. 5.

3.2. Hypertension

Cheng et al. revealed characteristic changes in SMC, EC, mesenchymal cells, and immune cells during the progression of hypertension through scRNA-seq of more than 20,000 cells in the aorta and mesenteric aorta of spontaneously hypertensive rats. Multiple

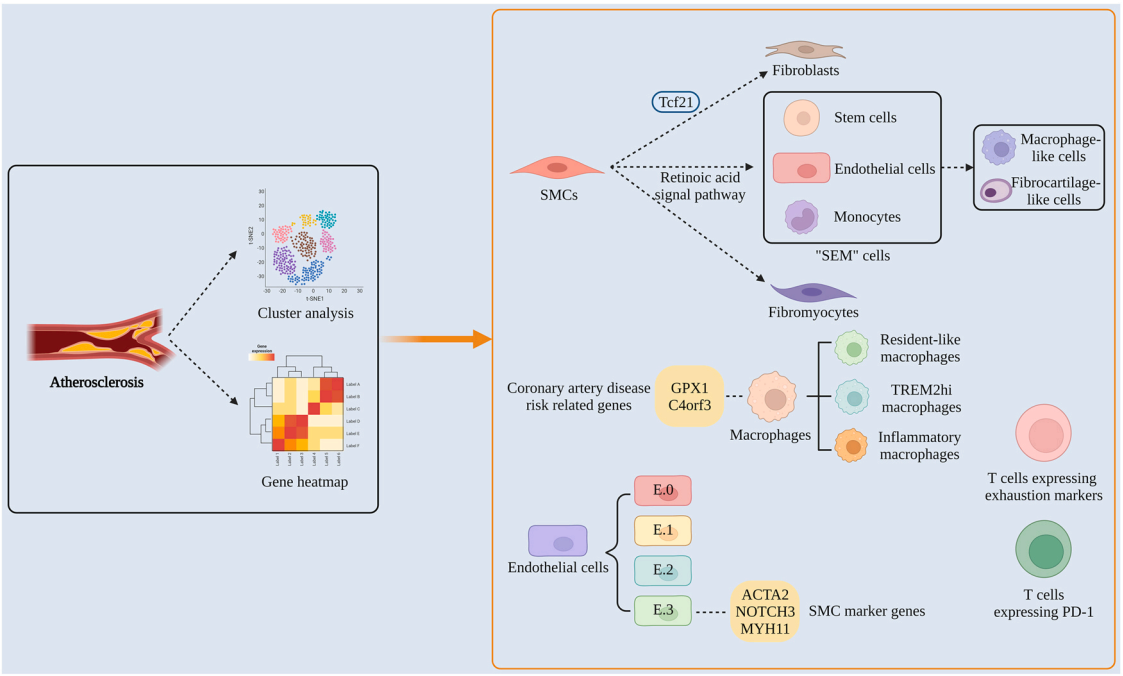

Fig. 5. Cellular and gene expression changes in atherosclerosis.

inflammatory cytokine genes were found to be upregulated in aortic cells, thus suggesting that inflammatory cytokine-induced enrichment of immune cells may be responsible for vascular remodeling during the progression of hypertension [91].

3.3. Myocardial infarction

MI involves multiple pathophysiological processes, including cardiac fibrosis, immune response, and myocardial regeneration [92]. The integration of scRNA-seq and ST in MI indicated significant differences between the transcriptional spectrum in MI and healthy hearts [93].

3.3.1. Cardiac fibrosis

Myocardial fibrosis in MI is a pathophysiological response that occurs in the heart after acute MI and involves multiple cell types [94]. Many myocardial cells die because of ischemia after acute infarction, thus resulting in a strong inflammatory response; the original myocardial tissue at the infarct region is replaced by ECM deposited produced by fibroblasts [95,96]. Through Tomo-seq, the expression of disease-associated genes such as Nppa (cardiac remodeling/hypertrophy), Col3a1 (fibrosis), and Atp2a2 (calcium handling) was discovered after MI, and the transcriptional regulator Sox9 was found to be involved in post-infarction cardiac fibrosis through regulation of ECM-associated gene expression [92]. Meanwhile, Sox9 was also indicated to participate in cardiac fibrosis by regulating profibrotic cardiomyocyte-fibroblast crosstalk [97]. Of note, cytoskeleton-associated protein 4 (CKAP4) was identified as a new marker of fibroblast activation during ischemic injury [98]. Besides, on days 3 and 7 after MI, a group of fibroblast lineages with strong anti-wingless-associated integration site (anti-WNT) transcriptomic signature was identified, and myofibroblast subtypes expressing antifibrotic transcriptional characteristics or profibrotic transcriptional characteristics were also observed [99].

Meanwhile, post-MI cardiac fibrosis is also an important cause of ventricular remodeling; the latter is manifested by ventricular enlargement and diminished ventricular ejection function and will affect the long-term prognosis of patients [100,101]. Villalba et al. identified and characterized the unique fibroblast subpopulations after MI by integrating bulk RNA-seq, scRNA-seq, and scATAC-seq. In this research, an activated fibroblast subpopulation of highly expressing recombinant human collagen triple helix repeat protein 1 (CTHRC1) shows prominent characteristics promoting fibrosis. CTHRC1 is a secretory protein that participates in collagen matrix synthesis through transforming growth factor-β (TGF-β) signaling. ST technologies have demonstrated that fibroblasts with high CTHRC1 expression are localized in scar tissue. And elevated mortality due to MI and diminished fibrosis reaction have been observed in CTHRC1-deficient mice. Thus, CTHRC1 was presumed to be a potential intervention target for ventricular remodeling and myocardial fibrosis in MI [102]. Similarly, ST analysis by Yamada et al. revealed high expression of the mechano-sensing gene Csrp3 in the MI border zone and suggested that Csrp3 may be a potential therapeutic target for the prevention of post-MI ventricular remodeling [103]. Besides, cardiomyocytes display upregulation of secretory factors such as beta-2 microglobulin (B2M) during myocardial ischemia-reperfusion injury, which in turn activate fibroblasts in a paracrine manner and participate in the fibrotic response during post-ischemic myocardial repair [104].

3.3.2. Immune response

The rapid activation of the innate immune system after MI produces a strong but transient inflammatory response. Neutrophils and macrophages express interferon-stimulated genes after MI, and this process begins in the bone marrow and is regulated by the transcriptional regulators Tet2, Irf3, and Nrf2 [105]. By combining human heart single-cell genomics, epigenomics, and ST data, Kuppe et al. established a spatial multi-omics map and observed interactions between macrophages and fibroblasts in the human heart after MI [106]. Dynamic changes in the proportions of 12 significant heart immune cell populations from the first day to the seventh day after MI were demonstrated by ST and scRNA-seq. It is noteworthy that the spatiotemporal heterogeneity of macrophages is closely associated with the cardiac repair, and the proportion of Trem2 macrophages with anti-inflammatory characteristics increases in the late stage of MI [107]. The immune cell subtypes and their dynamic

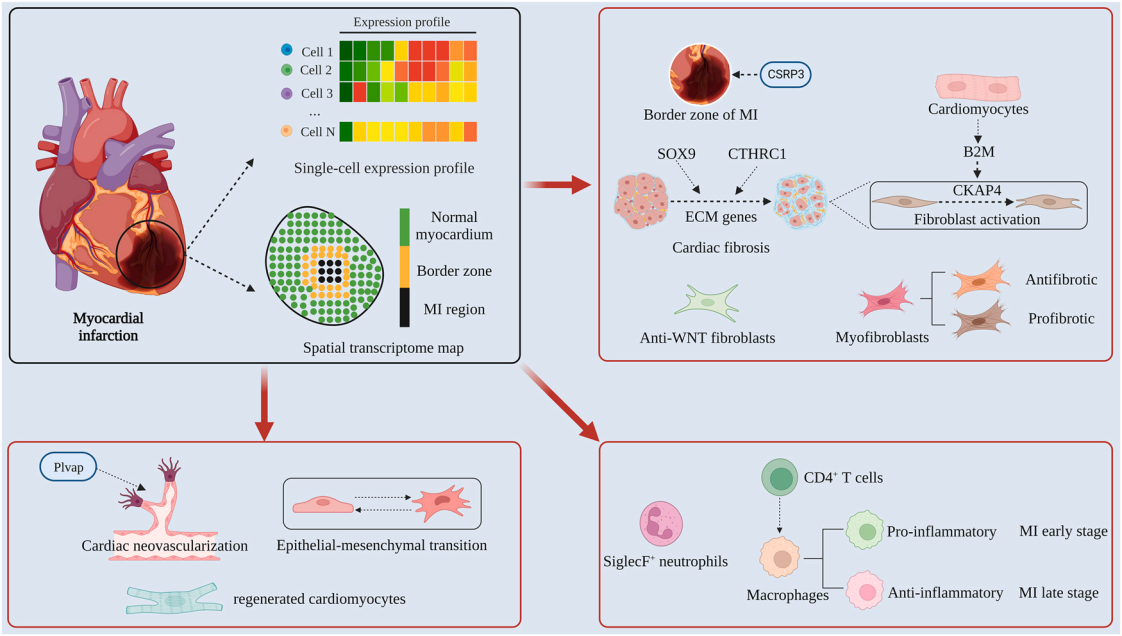

Fig. 6. Cellular and gene expression changes in MI.

changes at 0, 3, 7, and 14 days after MI were also described through scRNA-seq by Jin et al. The macrophages with the largest proportion peaked on the third day after MI, and the main subsets were Mø-5 and Mø-6, which expressed pro-inflammatory factors primarily [108]. Meanwhile, Calcagno et al. mapped the dynamics of neutrophil gene expression before and after MI and identified a subpopulation of neutrophils specifically expressing eosinophilic surface marker SiglecF, which was enriched in pro-inflammatory genes and displayed a high proportion over four days after infarction [109].

3.3.3. Vascular endothelial alteration

Previous scRNA-seq of ECs after MI has identified plasmalemma vesicle-associated protein (Plvap) as a potential target for cardiac neovascularization after MI [110]. To explore the molecular characteristics and phenotypic transformation of ECs after cardiac ischemic reaction, Tombor et al. performed scRNA-seq on mouse heart non-cardiomyocytes at 0, 1, 3, 5, 7, 14, and 28 days after MI and described the characteristics of cellular gene expression at different stages after MI. On the first day after MI, GO analysis of ECs indicated significantly upregulated expression of genes involved in the cell hypoxia response, positive regulation of the inflammatory response, programmed cell death, and angiogenesis. EMT, ECM, and cell proliferation gene expression are significantly enriched in ECs from day 1 to day 7 after MI. Cell cycle bioinformatics analysis confirmed the increased proliferation of ECs after MI. The number of ECs in the S phase increases on the 3rd day and returns to a steady state on the 14th day. Notably, EndMT may occur on a reversible continuum in response to hypoxia and inflammatory injury rather than being a permanent transition between differentiated cell states [79].

3.3.4. Myocardial regeneration

Zhang et al. performed scRNA-seq on post-MI regenerated cardiomyocytes and found that regenerated cardiomyocytes after MI display characteristics of dedifferentiation and upregulation of genes associated with the cell cycle and proliferation [111]. In addition, the gene expression of CD4 + T cells and CD8 + T cells is altered after myocardial injury. CD4 + T cells could participate in the regeneration process after myocardial injury by regulating the polarization process of profibrotic macrophages [112]. A brief summary of the findings is shown in Fig. 6.

3.4. Cardiomyopathy

The European Society of Cardiology defines cardiomyopathy as structural and functional abnormalities of the heart muscle which are not caused by coronary artery disease, hypertension, valvular disease, or congenital heart defects [113].

3.4.1. Pediatric mitochondrial cardiomyopathy

Hu et al. used sNucDrop-seq (a droplet microfluidics-based massively parallel single-nucleus RNA-sequencing method) to analyze the transcriptional spectrum of neonatal mouse heart cells in healthy and diseased states. Significant changes in the subtype composition, metabolic status, and function of several cell types (cardiomyocytes, fibroblasts, and endothelial cells) were observed in the hearts of mice with pediatric mitochondrial cardiomyopathy, such as down-regulation of the mitochondrial oxidative phosphorylation pathway and up-regulation of ribosome-associated genes. Moreover, the gene regulatory networks of GDF15 were constructed in different cell types, and the GDF15 used to be defined as a heart-derived hormone and an essential biomarker for heart disease diagnosis [114].

3.4.2. Hypertrophic cardiomyopathy

Dynamic changes in cardiac cell subtypes and the spatiotemporal interaction between cell types in different stages of hypertrophic cardiomyopathy (HCM) were demonstrated through scRNA-seq by Ren et al. Macrophage activation and subtype conversion are defined as critical events in the middle stage of cardiac hypertrophy and could be a potential therapeutic target for dapagliflozin, TD139, and arglabin [6]. Simultaneously, through bioinformatic analysis of snRNA-seq datasets from patients with obstructive HCM or non-obstructive HCM patients, Codden et al. found that patients with obstructive HCM and nonobstructive HCM show diminished intercellular communication caused by changes in integrin-β1 expression, but the decrease is more significant in obstructive HCM. Significant differences were observed in ECM interactions and immune cell activation between the two groups, as well as differential

regulation of adenylate cyclase and calcium channels. The results also provided potential targeting mechanisms for HCM therapy [115].

3.4.3. Dilated cardiomyopathy

The unfolded protein response pathway has been found to be protective in dilated cardiomyopathy (DCM) through scRNA-seq of human induced pluripotent stem cell-derived cardiomyocytes, thereby suggesting that the unfolded protein response may be a potential target pathway for DCM therapy [116]. Similarly, the transcription factor AEBP1 has been identified as a regulator of myocardial fibrosis in DCM, which is involved in the phenotypic remodeling process of fibroblasts; both macrophage-endothelial cell interaction and infiltration of immune cells such as T cells have been observed during myocardial fibrosis [117].

Interestingly, Chaffin et al. performed snRNA-seq on DCM, HCM, and normal hearts and observed consistent transcriptional profiles between DCM and HCM. The investigators also identified several genes potentially associated with cardiomyopathic fibroblast activation, including the ECM protein-encoding gene PRELP, COL22A1, and the metabolism-associated gene JAZF1. And the proportion of resident cardiac macrophages in both DCM and HCM was decreased [118].

3.4.4. Arrhythmogenic cardiomyopathy

Yuan et al. performed scRNA-seq on epicardial-derived cells in a mouse model of arrhythmogenic cardiomyopathy (ACM). Their findings indicated that epicardial-derived cardiac fibroblasts and epithelial cells are involved in pathological processes such as myocardial fibrosis and arrhythmias in ACM mouse models, by expressing paracrine factors mediating the EMT, including TGF-β1 and fibroblast growth factor [119]. Through Tomo-seq of ACM heart, Boogerd et al. found that ZBTB11 is specifically enriched in the myocardium and replaced by fibrous adipose fibro-fatty tissue, and subsequent experiments demonstrated that ZBTB11 induces autophagy and apoptosis of cardiomyocytes in ACM [120].

3.5. Myocarditis

Myocarditis involves multiple immune cells [121]. Hua et al. performed scRNA-seq of more than 34,000 CD45(+) cells in hearts with experimental autoimmune myocarditis. They revealed the transcriptional profiles of immune-inflammatory cells during the progression of myocarditis to cardiomyopathy. Hif1a may be involved in the myocardial inflammatory response by regulating macrophage and T-helper cell 17 activity, thereby participating in the myocardial inflammatory response, and may serve as a potential clinical target for myocarditis treatment [122]. At the same time, Mantri et al. constructed the viral myocarditis transcriptional profile through scRNA-seq and ST. Cytotoxic T cells were recruited by inflamed endothelial cells in myocarditis tissue, and the immune-mediated cell type-specific injury and stress responses were also observed [123].

3.6. Heart failure

Heart failure (HF) is the end-stage of many heart diseases, a complex pathophysiological process in which many cytokines and signaling pathways are involved [124].

3.6.1. Signaling pathways in HF cardiomyocytes

ScRNA-seq on mouse cardiomyocytes has indicated that cardiac hypertrophy can evolve into heart failure in the presence of sustained pressure overload, which involves myocardial remodeling caused by activation of the p53 signaling pathway and HF genes in the late stages of cardiac hypertrophy [125]. Through scRNA-seq and ST analysis, Ko et al. found that the Htra3-TGF-β-IGFBP7 pathway is crucial in regulating cardiomyocyte homeostasis and cardiac fibrosis. This pathway eventually leads to myocardial fibrosis and HF by inducing the transformation of cardiomyocytes to a secretory phenotype; these findings provided potential biomarkers and therapeutic targets for HF diagnosis [126]. Moreover, Yamaguchi et al. performed scRNA-seq on cardiac tissue from mice and humans with HF-associated ventricular arrhythmia and identified cardiomyocytes overexpressing dopamine D1 receptor (D1R) in both groups. Cardiac D1R may be a potential target for treating HF-associated ventricular arrhythmia [127].

3.6.2. Genes in HF cardiomyocytes

Asp et al. used the ST technique to analyze the left ventricular and right atrial appendage biopsies of three patients with HF with preserved ejection fraction (HFpEF). The researchers observed the expression of five known fetal marker genes (Nkx2–5, Gata4, Tbx20, Tbx5, and SSEA-1) and the HOPX gene in the HFpEF heart. And the reactivation of these genes may protect cardiomyocytes by inducing apoptosis resistance [128].

Meanwhile, the Nppa gene expression is elevated in patients with HF and is associated with the Nkx2–5 and Gtf2b transcription factors, which can be used as a diagnostic marker for HF [129]. Similarly, the expression of cardiomyocyte size-associated gene MYH7 increases during HF [130]. And age also shows cell-specific and disease-specific effects on gene expression in failing human hearts [131].

3.6.3. Non-cardiomyocytes in HF

Interestingly, the ACKR1$^+$ ECs and other non-cardiomyocytes are actively involved in regulating the cellular behavior of cardiomyocytes during HF and recovery [132]. And the activation of immune cells in the presence of myocardial stress overload was observed through scRNA-seq by Martini et al. Regulatory T cells (Tregs) play an immunosuppressive role in the HF myocardium, and inhibition of Tregs in the myocardium might increase cardiotoxicity. Notably, programmed cell death protein 1 (PD-1), selectively expressed by Tregs, might serve as an effective target for HF intervention [133]. Besides, Komai et al. reported the role of CD8 + T cell depletion in improving cardiac function by regulating macrophages, thereby promoting compensatory cardiac hypertrophy. CD8 + T cells play an essential role in myocardial adaptation to cardiac pressure overload in the early stages of HF by regulating the overexpression of the macrophage growth factor Areg, Osm, and IGF-1 protein genes [134].

As to monocytes, Abplanalp et al. performed the single-cell analysis of monocytes in the peripheral blood of patients with HF. Compared with HF without DNMT3A mutation, HF with DNMT3A mutation shows high expression of inflammatory genes (including interleukin IL, NLRP3 inflammasomes, and the macrophage inflammatory proteins CCL3 and CCL4) and T-cell immunoglobulin genes in peripheral blood mononuclear cells. The investigators suggested that DNMT3A gene mutations may be involved in the HF process by promoting monocyte inflammatory responses and monocyte-T-cell interaction [135]. Moreover, significant alterations have been observed in the monocyte to T cell ratio and cellular gene expression patterns with HF compared with healthy individuals [136].

As to fibroblasts, McLellan et al. described intercellular communication by scRNA-seq in a mouse cardiac fibrosis model induced by angiotensin II. They identified two unique subpopulations fibroblast-Cilp and fibroblast-Thbs4, which are key drivers of fibrosis. Sex-specific differences also existed in the transcriptional map of the heart, including cell abundance and gene expression levels [137].

3.7. Cardiac valve diseases

Xu et al. isolated 34,632 cells from the aortic valve tissues of four patients with calcified aortic valve disease and two healthy people. The cell cluster VEC-2 expressing endothelial valve cells marker genes SELE, IL1RL1, and PI3 might indicate a cell population transitioning from endothelial valve cells to interstitial cells. The EndMT process was confirmed in calcified aortic leaflets [138].

3.8. Aortic diseases

Pedroza et al. conducted scRNA-seq to explore the dynamic changes in SMC phenotype in the aortic tissues of Marfan syndrome model mice. They defined a specific SMC cluster with specific expression of ECM regulation and collagen synthesis-associated genes in adult Marfan syndrome mice. While the TGF-β and Klf4 genes potentially regulate the dynamic phenotypic changes in SMCs and ultimately promote the progression of aortic aneurysms [139]. Li et al. performed scRNA-seq on ascending aortic tissue from patients with ascending thoracic aortic aneurysm (ATAA) and identified 11 major cell types. And the differential gene analysis results suggested mitochondrial dysfunction in ATAA tissues compared with normal tissues [140].

ST analysis of the ascending aortic tissue from a patient with Stanford type A aortic coarctation has shown enrichment of aortic coarctation tissue for genes associated with hypertension and atherosclerosis, and the macrophages and stem cells predominantly distributed at the tear site [141]. Sawada et al. demonstrated the important role of second heart field-derived cells in aortic disease through scRNA-seq. A potential causal relationship has been reported between the cellular heterogeneity of second heart field -derived cells and aortic disease [142].

3.9. Congenital heart diseases

Congenital heart diseases are usually associated with genetic predisposition [143]. Through scRNA-seq of healthy fetal hearts and hearts with anti-SSA/Ro associated congenital heart block, Suryawanshi et al. found that the expression of interferon responses and ECM-associated genes in congenital heart block heart cells are greater than that in healthy fetal heart cells [144]. And Kathiriya et al. established a hypothetical gene regulatory network of congenital heart disease and identified the role of the transcription factor Tbx5 in the differentiation of human induced pluripotent stem cells to cardiac cells, according to an ST database [34]. The genetic interaction between Tbx5 and MEF2C was verified. Moreover, diminished expression of Tbx5 affects multiple developmentally important cardiac pathways, including genes associated with cardiac development, cardiomyocyte function, and congenital heart disease [145].

3.10. Other cardiac diseases

Walls et al. analyzed the changes in gene expression patterns and regional heterogeneity in heart tissue after targeted irradiation. Hydrogen peroxide metabolic process-associated gene expression is upregulated in the heart after targeted irradiation. The gene expression of Cav1, Tagln, Myh6, and C1qa is upregulated in atrial tissue, and these genes are associated primarily with the vascular system, cardiomyocytes, and the complement pathway. At the same time, Nppa and prostaglandin D2 synthase (Ptgds) genes are upregulated in ventricular tissue [146].

Vidal et al. used snRNA-seq to analyze the aging hearts in 12-week-old and 18-month-old mice. They found significant changes in the gene expression patterns of aged cardiac fibroblasts, such as upregulated expression of Serpine1 and Serpine2, which have anti-angiogenic effects. An increase in the proportion of fibroblasts expressing osteoblast genes at the epicardial level is associated with the progression of cardiac calcification [147]. Meanwhile, cell phenotype alteration from proliferation to secretion has been observed in cardiac scar formation during the maturation of the heart, together with loss of cell regenerative potential [43].

Porritt et al. described the immune cell landscape of the Kawasaki disease vasculitis mouse model by scRNA-seq and ST. A phenotypic switch of VSMCs induced by IL-1β signaling was observed in coronary arteries. Notably, NLRP3 was defined as a potential target for the pharmacological treatment of Kawasaki disease cardiovascular inflammation [148]. Interestingly, by scRNA-seq and ST, the unique structure of GPNMB+ (transmembrane glycoprotein NMB) multinucleated giant cells adjacent to human leukocyte antigen–DR isotype+ epithelioid was defined as the feature of human cardiac sarcoidosis, compared with other inflammatory cardiac diseases like giant cell myocarditis and lymphocytic myocarditis [149].

4. Summary and outlook

This review summarized scRNA-seq and ST findings in cardiac development and disease. From the perspective of basic medicine, multiple anatomical structures with diverse functions are formed during heart development through a process involving the participation of many genes, transcription factors, and signaling pathways [150,151]. ScRNA-seq can identify specific altered cell proportions (e.g., neutrophils, endothelial cells, or fibroblasts), thereby indicating cellular phenotypic transition during cardiogenesis and cardiac disease, and can demonstrate cell developmental trajectories and intercellular communication [152]. ST allows for visual comparison of the gene expression profiles of different anatomical structures of the heart (e.g., atria and ventricles) in the spatial dimension [153–155]. The integration of transcriptomic technologies, such as scRNA-seq and ST, provides an important data source for understanding the key regulators of cardiac development and the pathophysiological mechanisms of cardiac disease.

From the perspective of clinical medicine, the primary treatment modalities for human heart disease currently include drug therapy and interventional procedures [156]. ScRNA-seq and ST technologies can be applied for translation from basic research to clinical diagnosis and treatment. Based on the mammal or clinical human heart samples reflecting different ages, sexes, and diseases, researchers can construct a spatiotemporal transcriptional database of the heart before and after drug and interventional treatments by scRNA-seq and ST. For chronic heart diseases (e.g., hypertension, chronic heart failure), changes in transcriptional profiles after pharmacological interventions will aid in exploring individualized therapeutic targets and developing targeted drugs. The dynamic changes in cell proportions and gene expression in the infarcted region after MI will also contribute to the prognostic assessment of patients. However, current research on scRNA-seq and ST application in arrhythmia diseases (such as atrial fibrillation, sinus node dysfunction, atrioventricular block, and supraventricular tachycardia) is insufficient.

On the other hand, scRNA-seq still has limitations in cardiology applications, from laboratory and clinical patient sample extraction to computer visualization analysis algorithms [157]. First, tissue samples for scRNA-seq are more often derived from experimental animals (e.g., mice, rats, chickens). The process of obtaining idealized human heart tissue in healthy or diseased states is restricted due to the specificity of the samples and the ethical principles of clinical medical research. Second, sample handling procedures (e.g., tissue preservation and tissue dissociation) still need to be optimized to minimize cell loss and improve the accuracy of the final analysis results. Third, the existing library preparation, sequencing depth, and data quality control process may also result in the loss of potentially important genetic information data [158]. Fourth, as the

content of human single-cell databases continues to expand, algorithms for bioinformatics analysis (e.g., dimensionality reduction and cell trajectory analysis) need to be further optimized to improve accuracy [159]. Finally, the high cost of sequencing and the complex bioinformatics codes make it difficult for potentially interested cardiovascular researchers to participate. For ST, there is the same limitation as for scRNA-seq regarding human heart sample acquisition. In addition, transcriptional profiling of a single spot in ST may include multiple types of cells [160].

With the improvement of bioinformatics technologies and databases, it is foreseen that single-cell multi-omics (including single-cell transcriptomics, ST, metabolomics, and proteomics) will provide a more comprehensive understanding and advanced microscopic definitions of cardiac physiological and pathological processes. Meanwhile, combining single-cell transcriptome technology with machine learning, clinical trials, and cohort studies in hospitals enables the exploration of novel diagnostic and therapeutic approaches to hereditary diseases and rare diseases, advancing the development of precision medicine.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

CRediT authorship contribution statement

Xianglin Long: Conceptualization, Writing – original draft, Writing – review & editing. **Xin Yuan:** Writing – review & editing, Methodology. **Jianlin Du:** Supervision, Conceptualization, Writing – review & editing. All authors contributed to the article and approved the submitted version.

Consent to publish

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Data Availability

Not applicable.

Declaration of Competing Interest

The authors declare no conflict of interest. Figures were created with BioRender.com.

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