

Heterogeneity and characteristic changes in cardiomyocytes and non-cardiomyocytes following myocardial infarction: Insights from single-cell sequencing analysis (Review)

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Abstract. Myocardial infarction (MI) is an important pathological event in cardiovascular disease and the heterogeneous changes in cardiomyocytes and non-cardiomyocytes that occur following its occurrence have a profound effect on cardiac repair and functional recovery. Single-cell sequencing, a novel technology for the analysis of tissues at the single-cell level, permits a comprehensive insight into the heterogeneity of different cell populations. The current review presented a summary of the application of single-cell sequencing to detect the heterogeneity of cardiomyocytes and non-cardiomyocytes following MI. It focused on the classification and changes of cell clusters and explored the mechanisms of post-infarction cardiac regeneration and remodeling with the aim of providing a theoretical foundation for identifying potential targets to enhance the diagnosis and treatment of post-infarction cardiac remodeling.

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1. Introduction

Myocardial infarction (MI) is associated with a high incidence of morbidity and mortality. Despite the beneficial effects of prompt reperfusion therapy on survival rates in the initial stages of MI, post-MI cardiac remodeling remains a significant contributing factor to heart failure and later mortality (1). Cardiac remodeling is a complex process involving functional and structural disturbances in the myocardium, primarily driven by myocardial injury and overload (2). This process is typically characterized by three distinct stages: Inflammation, proliferation and maturation (3). Single-cell RNA sequencing (scRNA-seq) represents a novel technological advancement that facilitates transcriptome analysis at the single-cell level, thereby offering comprehensive insights into the heterogeneity of diverse cell populations within tissues. This technology enables the identification of novel marker genes, cellular clusters and cellular heterogeneity, as well as the inference of cellular lineage trajectories. Furthermore, it can elucidate intercellular interactions and facilitates comparisons between healthy and diseased cells (4,5). In the context of MI, scRNA-seq can elucidate transcriptional variability among similar cell types in post-infarction tissues in comparison to normal tissues. Furthermore, it can detect gene expression differences and dynamic changes over time.

The present study reviewed the application of scRNA-seq for the analysis of temporal changes in cardiomyocytes and non-cardiomyocytes following MI. It aimed to provide a theoretical basis for identifying new therapeutic targets for preventing and treating post-infarction cardiac remodeling by classifying different cell clusters and elucidating their roles in post-infarction tissue remodeling.

2. Single-cell sequencing

Emergence of single-cell sequencing. Traditional gene expression analysis techniques, such as quantitative PCR, microarrays

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and high-throughput RNA sequencing, permit the analysis of cell populations that are heterogeneous at the gene expression level. These methods provide only an averaged representation of gene expression across the group, often overlooking low-abundance transcripts and masking the unique characteristics of individual cells. As a result, intercellular heterogeneity remains largely undetected (6-8). To address these limitations, single-cell sequencing technology was developed, enabling a shift from population-level analyses to the study of individual cells. This technological advancement allows for the in-depth exploration of tissues, organs and even entire systems at the cellular level, markedly enhancing the current understanding of cellular diversity and function (9-11).

Single-cell sequencing technology. Single-cell sequencing technology can be broadly summarized as the amplification and sequencing of DNA or RNA at the level of a single cell. The process primarily involves single-cell isolation, nucleic acid processing, DNA amplification, library construction, sequencing and data analysis (12-15). Currently, single-cell sequencing techniques are categorized into several types, including single-cell genome sequencing, single-cell transcriptome sequencing (scRNA-seq), single-cell epigenome sequencing, single-cell multiomics sequencing and spatial transcriptome sequencing. Common single-cell sequencing methods include Smart-seq (16), Smart-seq2 (17), Drop-seq (18), STRT-seq (19,20), MARS-seq (21), MARS-seq2 (22) and Fluidigm C1 (23), which can be selected based on specific experimental goals. Single-cell sequencing has been applied across various fields, such as stem cell and developmental biology, oncology and immunology and has become increasingly important in both clinical and basic medical research. The present review focused on the application of single-cell sequencing for analyzing individual cells within post-infarction myocardial tissues. Specifically, it highlighted the exploration of the heterogeneity and enrichment of cardiomyocytes and non-cardiomyocytes in these tissues, demonstrating the clustering of these cells at different time points (for example, days 3, 7, 8 and 21 post-infarction) compared with control groups.

Advantages and disadvantages of different single-cell sequencing methods to study cardiomyocytes. Cardiomyocytes in the heart can reach $\leq 100\ \mu\text{m}$ in length and $25\ \mu\text{m}$ in width. This presents challenges for current commercial single-cell transcriptome sequencing (scRNA-seq) platforms, which have limitations regarding cell size. For instance, the 10X Genomics platform has a microtubule diameter of $50\ \mu\text{m}$ in its chip and it is recommended that the captured cells not exceed $40\ \mu\text{m}$. As a result, cells must be filtered using a $30\text{--}40\ \mu\text{m}$ sieve to remove larger cells prior to capture. However, this filtration step excludes large cardiomyocytes and prevents the generation of scRNA-seq data from adult primary heart tissues (24). To address this issue, the majority of studies on cardiomyocytes have adopted single-nucleus RNA sequencing (snRNA-seq), as it targets the nuclei of cells rather than the entire cell. While snRNA-seq offers the advantage of detecting nuclear-localized transcripts and maintaining membrane integrity, it has limitations. Specifically, the method reduces assay sensitivity and omits cytoplasmic mRNA information. Additionally, isolated

nuclei tend to adhere more than isolated whole cells, increasing the risk of aggregation and duplex rate expansion (25,26). However, snRNA-seq allows for the transcriptomic analysis of tens of thousands of nuclei isolated from fresh or frozen tissues, including mature mammalian hearts. It facilitates the isolation of intact single cells from complex tissues, reduces bias from easily recoverable cell types and mitigates aberrant gene expression during cytokinesis (27,28).

3. Single-cell sequencing to analyze changes in cardiomyocytes following MI

Cluster analysis and changes in cardiomyocytes following MI. There are two key studies that focus on the cluster analysis of cardiomyocytes following MI, as outlined in Table I. In 2020, Litviňuková *et al* (29) identified five ventricular cardiomyocyte populations (vCM1-vCM5) and five atrial cardiomyocyte populations (aCM1-aCM5) by isolating single cells, nuclei and CD45⁺ cells from the right and left ventricular free walls, right and left atria, left ventricular apices and interventricular septum using both scRNA-seq and snRNA-seq. Detailed information about the marker genes for each cluster is provided in Fig. 1A. vCM1 and vCM2 showed minimal differences, with vCM2 highly expressing PRELID2 and the myosin genes MYH6 and CDH13. vCM3 expressed stress-responsive genes, including ANKRD1 (30), FHL1 (31), DUSP27 (32), XIRP1 and XIRP2. vCM4 was enriched in nuclear-encoded mitochondrial genes such as NDUFB11, NDUFA4, COX7C and COX5B. vCM5 highly expressed DLC1 and EBF2. Regarding the atrial myocytes, aCM1 expressed basal atrial myocyte genes and lower levels of neurological function molecules such as ADGRL2, NFXL1 and ROBO2, while aCM2 was enriched in the right atrium and predominantly expressed HAMP, SLIT3, ALDH1A2 (33), BRINP3 and GRXCR2. aCM3-5 and vCM3-5 shared similar transcriptional profiles. The present study systematically mapped the molecular typing of atrial and ventricular myocytes for the first time in adults, laying the foundation for understanding cellular diversity in the physiological state of the heart.

Cui *et al* (34) performed snRNA-seq on both regenerating and non-regenerating cardiomyocytes in neonatal mice on postnatal days 1 and 8, as well as on days 1 and 3 following MI [1 and 3 days post-infarction (dpi)], comparing these with sham-operated mice. The authors identified five main cardiomyocyte populations, named CM1-CM5, based on the expression of Myh6. The marker genes for each cluster are detailed in Fig. 1B. CM2 was characterized by high expression of cell cycle genes such as Top2A and Cdc5. CM4 highly expressed metabolic genes involved in oxygen reduction (such as Atp5b, Sod2 and Mb) and mechanistically, overexpression of NFYA and NFE2L1 promoted cardiomyocyte proliferation and survival through the activation of CM4-associated injury response pathways. CM5 exhibited high expression of actin filament-regulated genes (such as Xirp2, Ankrd1 and CD44) with a gene expression profile suggestive of decreased cardiac function (downregulation of contractile/calcium channel genes), activation of stress/apoptotic pathways and association with pathological remodeling (upregulation of dilated cardiomyopathy and muscle hypertrophy-related

Table I. A typical study on the analysis of myocardial cell subsets by ScRNA-seq.

Source of organization	Right and left ventricular free walls, right and left atria, left ventricular apex and interventricular septum in adults	Ventricular tissue in mice
Methods	1. Fresh tissue for ScRNA-seq 2. Frozen tissue for SnRNA-seq	SnRNA-seq
Number of cells captured	45,870 cells; 78,023 CD45 ⁺ enriched cells and 363,213 nucleus	21,737 myocardial cell nucleus
Clusters	vCM1-vCM5; aCM1-aCM5	CM1-CM5

Data from Litviňuková *et al* (29) and Cui *et al* (34) (all from 2020 literature specifically analyzing cardiomyocyte by single-cell sequencing and have impact factors >8 points and all have identified different cardiomyocyte clusters and explored associated functions and the experiments were conducted using publicly available datasets). ScRNA-seq, single-cell transcriptome sequencing; SnRNA-seq, single-nucleus RNA sequencing; vCM, ventricular cardiomyocyte clusters; aCM, atrial cardiomyocyte clusters.

genes). Additionally, CM4 was found to be associated with cardiac regeneration, as it upregulated cell cycle genes, particularly during the G₂/M phase. The proportion of CM4 cardiomyocytes increased following cardiac injury, with a marked increase in regenerating hearts at 3 dpi, suggesting that CM4 may be a key source of cardiomyocyte proliferation after injury. By contrast, the CM5 population was markedly increased in non-regenerating hearts at 1 and 3 dpi, which was potentially linked to hypertrophic remodeling and cardiomyocyte apoptosis after infarction (34). Together, the aforementioned two studies revealed significant molecular heterogeneity in cardiomyocytes and identified key functional subpopulations. In particular, the study of Cui *et al* (34) highlighted the value of CM4 as a potential target for regenerative therapies, as well as the importance of CM5 as a marker of pathological remodeling.

Single-cell sequencing to investigate the regenerative properties of cardiomyocytes. Kretzschmar *et al* (35) found that circulating cardiomyocytes were observed only during the early postnatal growth phase by using single-cell mRNA sequencing and genetic lineage tracing in two Ki67 knock-in mouse models. While a large number of proliferating cardiomyocytes were detected in neonatal hearts, they were absent in damaged adult hearts. Similarly, Cui *et al* (34) analyzed the number of proliferating cardiomyocytes in regenerating hearts at 1, 3 and 7 dpi, revealing that cardiomyocyte proliferation commenced as early as 3 dpi.

In other studies, fluorescence-activated cell sorting was used to isolate cardiomyocyte nuclei with an antibody targeting the myocardial nuclear membrane protein PCMI, which then facilitated snRNA-seq analysis of cardiomyocyte nuclei (24,36,37). Analysis of nuclear RNAs revealed that 23% of unique molecular identifiers were mapped to introns and 52% to exons, confirming cardiomyocyte regeneration following MI in neonatal mice (34). Additionally, Tani *et al* (38) identified the mechanisms underlying cardiac reprogramming for *in vivo* repair through microarray and scRNA-seq.

That study demonstrated that cardiac reprogramming could repair chronic MI by promoting myocardial regeneration and reducing fibrosis.

4. Heterogeneity of non-cardiomyocytes and changes following MI

Endothelial cell (EC) heterogeneity and changes following MI. The heterogeneity of ECs following MI has been extensively studied using single-cell sequencing, with three key articles providing valuable insights, as summarized in Table II. In 2020, Wang *et al* (39) performed scRNA-seq on ventricular tissues from timed-pregnant ICR/CD-1 regenerating and non-regenerating mice, as well as age-matched sham controls, at 1 and 3 days following MI. Their analysis revealed that neonatal 1- and 8-days mouse hearts exhibited heterogeneous EC clusters. A total of six EC clusters were identified through cluster analysis, namely Art.EC, VEC1-3, Endo.EC and Pro.EC. The marker genes for each subcluster are shown in Fig. 2A. However, changes in the number of post-infarction subclusters were not discussed in detail. All six clusters exhibited high expression of the EC marker genes Cdh5 and Pecam1. The Cxcl12 gene and others were enriched in the Art.EC population (40), while H19 and Cpe were enriched in the Endo.EC population (41,42). Enrichment of cell cycle genes (Hmgb2, Birc5) in Pro.EC may provide a cellular source for vascular regeneration.

In the VEC clusters, Gpihbp1 was highly expressed, with VEC3 showing a higher expression level among the three subclusters (VEC1-3) (40). The VEC2 cluster overexpressed Cxcl1 and Icam1 and activated the ROS/TNF signaling pathway to drive an inflammatory response.

In 2019, Farbehi *et al* (43) conducted sham and MI surgeries on mice at 3 and 7 days post-infarction. The authors extracted the cardiac interstitial cell population from 8-week-old male PdgfraGFP/+ mice and performed single-cell expression profiling. A total of three major EC clusters (EC1, EC2 and EC3) were identified. The EC1 population expressed Ly6a

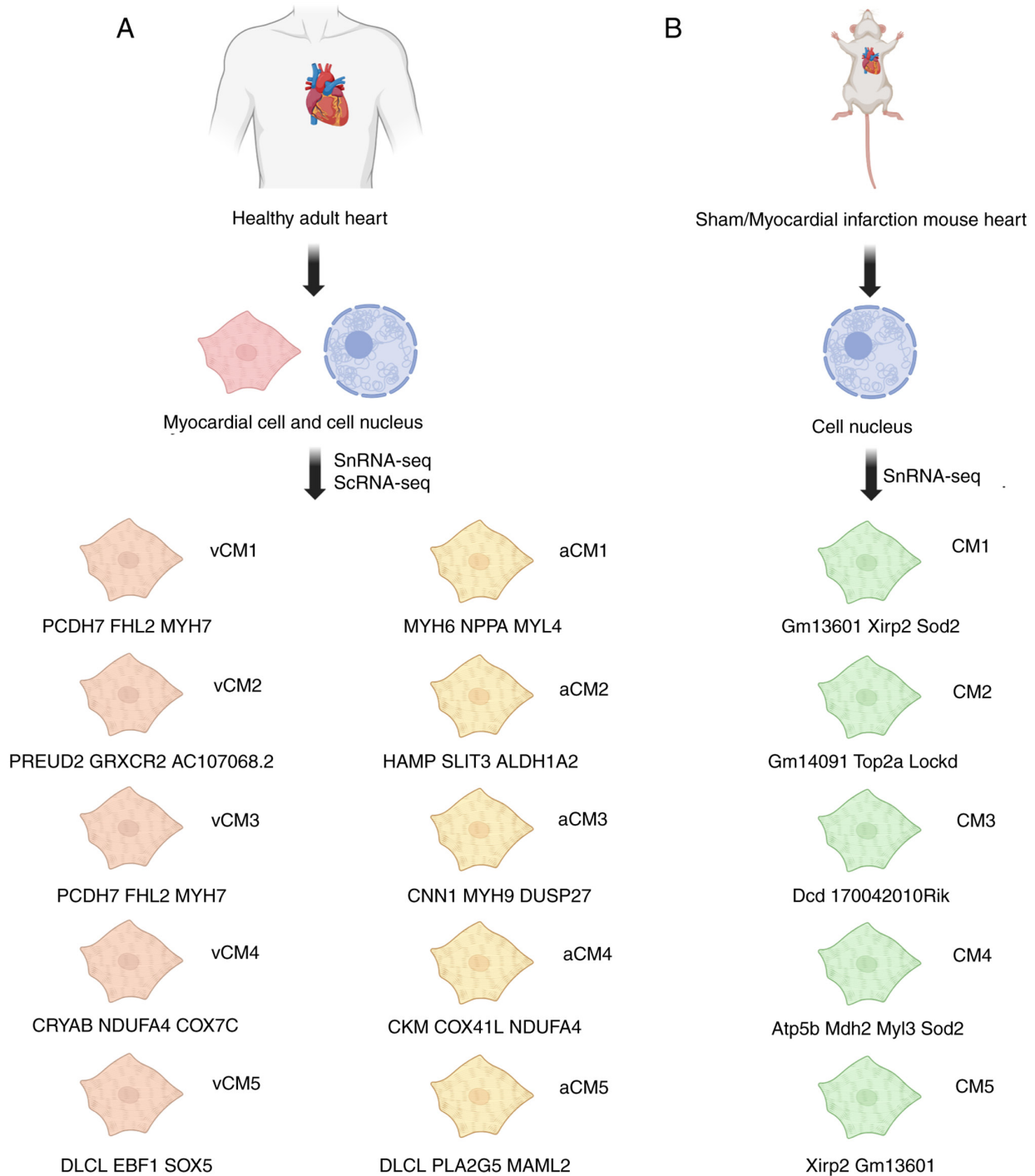


Figure 1. Cardiomyocyte cluster typing and marker genes for each cluster. vCM1-vCM5 represent myocardial cell populations under different ventricular conditions, which are determined by characteristics such as stress response, metabolic changes and proliferation. Marker genes such as MYH6 are notably expressed in vCM2, indicating that they are closely related to metabolic activities in myocardial repair. (A) Adult cardiomyocyte cluster typing and marker genes for each cluster. (B) Mouse cardiomyocyte cluster typing and marker genes for each cluster. SnRNA-seq, single-nucleus RNA sequencing; ScRNA-seq, single-cell RNA sequencing; vCM, ventricular cardiomyocyte clusters; aCM, atrial cardiomyocyte clusters; vCM1, functionally maintained cardiomyocytes; vCM2, myocardial cells enriched in the right ventricle (with high expression of MYH6); vCM3, stress-responsive cardiomyocytes (expressing ANKRD1, etc.); vCM4, high-energy metabolic cardiomyocytes (enriched mitochondrial genes, CRYAB); vCM5, electrophysiologically associated cardiomyocytes (high expression of DLCL and EBF2); aCM1, basic functional cardiomyocytes; aCM2, metabolic regulation of cardiomyocytes in the right atrium (predominantly expressed ALDH1A2, etc.); aCM3, smooth muscle-like cardiomyocytes; aCM4, cardiomyocytes with high metabolic activity; aCM5, electrophysiological regulation of cardiomyocytes; CM1, steady-state contractile mature cardiomyocytes; CM2, injury stress-response cardiomyocytes (high expression of Top2A and Casc5); CM3, metabolism-adaptive cardiomyocytes; CM4, inflammatory/fibrotic regulatory cardiomyocytes (expressing Atp5b, Sod2 and Mb); CM5, terminally differentiated/senescent cardiomyocytes (expressing Xirp2, Ankrd1 and CD44).

(encoding SCA1) and the vascular transcription factor Sox17, as indicated by marker genes, which may represent microvascular ECs. EC2 expressed Sox17 and Hey1, which function

downstream of the Notch signaling pathway and are important for arterial ECs. In addition to the marker genes, a number of EC3 cells expressed Prox1 and Lyve1. The EC1 cluster showed

Table II. A typical study on ScRNA-seq of endotheliocyte subsets.

Source of organization	Ventricular tissue of mice	Cardiac interstitial cell populations in mice	Non-cardiac muscle tissue from mice
Methods	10X Genomics scRNA-seq	10X Genomics ScRNA-seq	ScRNA-seq
Number of cells captured	17,320 cells	13,331 cells	35,312 cells
Clusters	Art.EC, VEC1-3, Endo.EC, Pro.EC	EC1, EC2, EC3	5 endothelial cell clusters

Data from Wang *et al* (39), Cui *et al* (34) and Tombor *et al* (44) (all from 2020-2021 literature specifically analyzing cardiac endothelial cells by single-cell sequencing with an impact factor of >6 points and all of them identified different clusters of endothelial cells and explored associated functions and the experiments were conducted using publicly available datasets). ScRNA-seq, single-cell transcriptome sequencing Art.EC, arterial ECs; VECs, vascular ECs; Endo.EC, endocardial ECs; EC, endothelial cell, Pro.EC, proliferating endothelial cell.

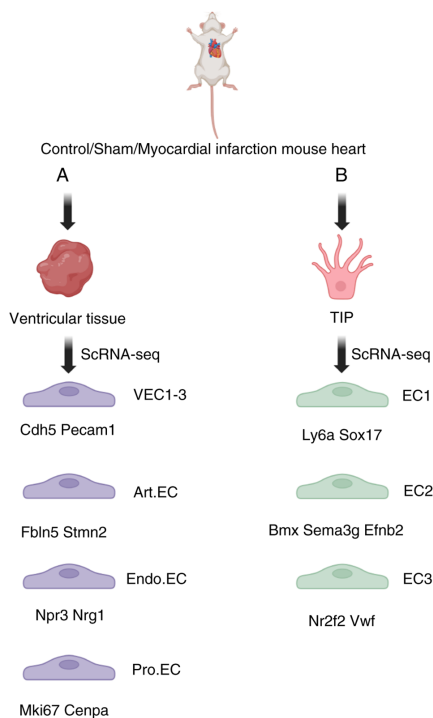


Figure 2. Endothelial cell cluster typing and marker genes for each cluster. The high expression of capillary marker *Gpihbp1* in VEC1 and VEC2, as well as the enrichment of macrovascular genes such as *Plvap* and *Vwf* in VEC3, indicate that they are related to the maintenance of vascular activity and angiogenesis. Endothelial cells have multiple functions in blood vessels, including vascular stability, blood flow regulation, material exchange and immune response. Following myocardial infarction, endothelial cells have the functions of repair, vascular regeneration and remodeling, as well as coagulation. (A) Endothelial cell clusters of ventricular tissue origin and marker genes. (B) Endothelial cell clusters of TIP origin and marker genes. TIP, cardiac interstitial cell population; ScRNA-seq, single-cell RNA sequencing; VEC1-3, venous endothelial cells (VEC1 highly expresses *Gpihbp1*, VEC2 cluster highly expresses *Cxcl1* and *Icam1*, VEC3 highly expresses *Plvap* and *Vwf*); Art.EC, arterial endothelial cells (enriched with artery-related genes such as *Cxcl12*); Endo, metabolism-related endothelial cells (specifically expressing metabolic genes such as *H19* and *Cpe*); Pro.EC, proliferative active endothelial cells [highly expressing proliferation genes *Hmgb2* and *Birc5*]; VEC1, microvascular endothelial cells [expressing *Ly6a* (encoding *SCA1*) and vascular transcription factor *Sox17*]; VEC2, arterial endothelial cells (involved in NOTCH signaling pathways, such as *Sox17*, *Hes1*); VEC3, venous endothelial cells.

a rapid decrease at 3 dpi and a subsequent increase at 7 dpi, although it remained below control levels.

In 2021, Tombor *et al* (44) conducted single-cell sequencing and trajectory analysis on non-myocardial tissues from C57BL/6J *Cdh5-CreERT2* mice at various time points (days 0, 1, 3, 5, 7, 14 and 28 post-infarction). The authors identified five EC clusters, with *Cdh5* and *Pecam1* serving as key marker genes. Subcluster 3 activated neutrophil chemotaxis and cytokine signaling to exacerbate inflammatory damage. Subcluster 4 showed an increase in number between days 1 and 7, returning to baseline levels after day 14. This subcluster expressed mesenchymal genes and proliferation markers, while fatty acid metabolism genes were downregulated, suggesting dysregulated energy metabolism.

Trajectory analysis confirmed that ECs underwent an 'interstitial' transient transition with partial recovery after 14 days. A separate study (45) found that *circFndc3b* was markedly downregulated in heart following MI. However, overexpression of *circFndc3b* in cardiac ECs increased vascular endothelial growth factor expression, enhanced angiogenic activity and reduced apoptosis in cardiomyocytes and ECs. Additionally, the LPA-LPA2 signaling pathway was shown to promote vascular neogenesis and maintain vascular homeostasis, which is essential for restoring blood flow and repairing ischemic tissues (46). EC clusters show a sequential response of inflammation, proliferation and mesenchymalization following MI and targeted intervention of specific subpopulations (including inhibition of VEC2 inflammation, amplification of Pro.EC and blockade of mesenchymal transformation) is expected to break through the current bottleneck in vascular regeneration therapy.

Fibroblast heterogeneity and associated changes following MI. Cardiac fibroblasts are transformed into myofibroblasts (MYOs) following injury, which plays a critical role in mediating healing after acute MI (AMI) and contributes to long-term fibrosis in chronic disease (47). Fibroblasts are essential for cardiac remodeling following MI. Several studies have analyzed the characterization and changes in various fibroblast clusters following MI using single-cell sequencing, as detailed in Table III.

Table III. A typical study on ScRNA-seq of fibroblast subsets.

Source of organization	Cardiac interstitial cell populations in mice	Non-cardiomyocytes from mice	Epicardial interstitial cells in mice	Ventricular tissue in mice	Ventricular tissue in mice	Ventricular tissue in mice	Mouse heart tissue
Methods	10X Genomics ScRNA-seq	ScRNA-seq	10X Genomics ScRNA-seq	10X Genomics ScRNA-seq	10X Genomics ScRNA-seq	ATAC-Seq	10X Genomics ScRNA-seq
Number of cells captured	13,331 cells	27,349 cells	38,600 cells	17,320 cells	10,487 cells	29,176 cells	-
Clusters	MYO, F-Act, F-Cyc, F-CI, F-SH, F-SL, F-WntX, F-Trans	Fibro-1, Fibro-2, Fibro-3, Fibro-4, Fibro-5, Fibro-Myo	I, II, III; (three main Myofb clusters: Myofb, ProlifMyofb, MFCs)	FB1-FB4, Pro.FB	CF1-7	A-J	F-SL, F-SH, F-Trans, F-WntX, F-Act, F-CI, F-Cyc, F-IFNS

Data from Nona *et al* (43), Zhang *et al* (51), Forte *et al* (53), Wang *et al* (39), Tani *et al* (38), Ruiz-Villalba *et al* (52) and Janbandhu *et al* (62) (all from the 2019-2023 literature analyzing cardiac fibroblasts exclusively by single-cell sequencing and with impact factors fluctuating from 2.9-38.6 points, all of which identified different clusters of fibroblasts and explored associated functions and the experiments were conducted using publicly available datasets). ScRNA-seq, single-cell transcriptome sequencing MYO/Myofb/myo, myofibroblasts; F-Act, activated fibroblasts; F-Cyc, a unique GFP⁺ proliferating population; F-CI, fibroblasts-circulating intermediates; F-SH, fibroblast Sca1 high cluster; F-SL, fibroblast Sca1 low cluster; F-WntX, fibroblast-Wnt-expressing cluster; F-Trans, fibroblast-transitional cluster; F-IFNS, IF stimulated; Pro.FB, proliferating fibroblasts; FB, fibroblast; ProlifMyofb, proliferative myofibroblasts; MFCs, a group of stromal fibroblasts; CF, cardiac fibroblasts; Fibro, fibroblast.

In 2019, Farbehi *et al* (43) classified the fibroblast population into eight clusters: MYOs, activated fibroblasts (F-Act), a unique GFP⁺ proliferating population (F-Cyc), fibroblasts-circulating intermediates (F-CI), fibroblast Sca1 high cluster (F-SH), fibroblast Sca1 low cluster (F-SL), fibroblast-Wnt-expressing cluster (F-WntX) and fibroblast-transitional cluster (F-Trans). The marker genes for each cluster were not detailed.

MYOs expressed fibrillogenic proteins, such as periostin (Postn) and contractile proteins such as smooth muscle actin (48), along with collagen genes, including Colla1, Col3a1 and Col5a2 (49), which can drive collagen deposition with scarring, with a significant increase in numbers on day 7 post-MI. F-Cyc specifically expressed the cell cycle genes Cdk1 and Mki67 (Ki67), a source of reparative cells that could inhibit overproliferation through targeted regulation, which peaked on day 3 and decreased on day 7 following MI, which is consistent with previous studies showing that the peak of fibroblast proliferation occurs on days 2-4 following MI (48,50). F-CI showed upregulated expression of genes involved in fibroblast activation, such as Postn, Cthrc1 and Acta2, although it did not express cell cycle markers, which is suggestive of a potentially activated fibroblast population that could enter the cell cycle. This cluster also exhibited upregulated protein translation genes, a feature not observed in F-Act. High expression of Ly6a (Sca1) and Pdgfra was observed in F-SH, with multidirectional differentiation capacity or involved in tissue repair, which was reduced on day 3 and partially restored on day 7 following MI. The newly identified F-WntX and F-Trans were present in both

pseudo and myocardial infarcted hearts and F-WntX specifically showed overexpression of the Wnt signaling pathway inhibitor Wif1, which affected the repair process and it was markedly reduced at day 7 post-MI.

A single-cell sequencing study by Zhuang *et al* (51) involving non-cardiomyocytes from healthy and post-infarction male mice (aged 8-10 weeks) at days 0, 3 and 7 identified six fibroblast clusters named Fibro-1, Fibro-2, Fibro-3, Fibro-4, Fibro-5 and Fibro-Myo. Marker genes for Fibro-4 and Fibro-5 were not markedly expressed. Marker genes for the remaining subgroups are shown in Fig. 3A. The proportion of fibroblasts decreased at 3 dpi compared with the sham group, while Fibro-Myo markedly increased at 7 dpi relative to other fibroblast clusters, indicating its crucial role in ischemic response and healing process. Cthrc1 and Ddah1 were both markedly upregulated in the Fibro-Myo cluster. Cthrc1 is a known marker for myofibroblasts (52), while Ddah1 can also serve as a myofibroblast marker. Thus, Fibro_Myo plays an important role in promoting cardiac healing and may act as a relevant subpopulation of intervening cells after improved cardiac remodeling.

In 2020, Forte *et al* (53) performed scRNA-seq on epicardial mesenchymal cells from seven male Wt1Cre transgenic mice, identifying three fibroblast populations (Type I, II and III). The marker genes for each population are shown in Fig. 3B. The marker gene ZsGreen was expressed in all Type I-III fibroblasts. The ratio of Type I fibroblasts was markedly higher on days 1 and 3 post-infarction, with the highest

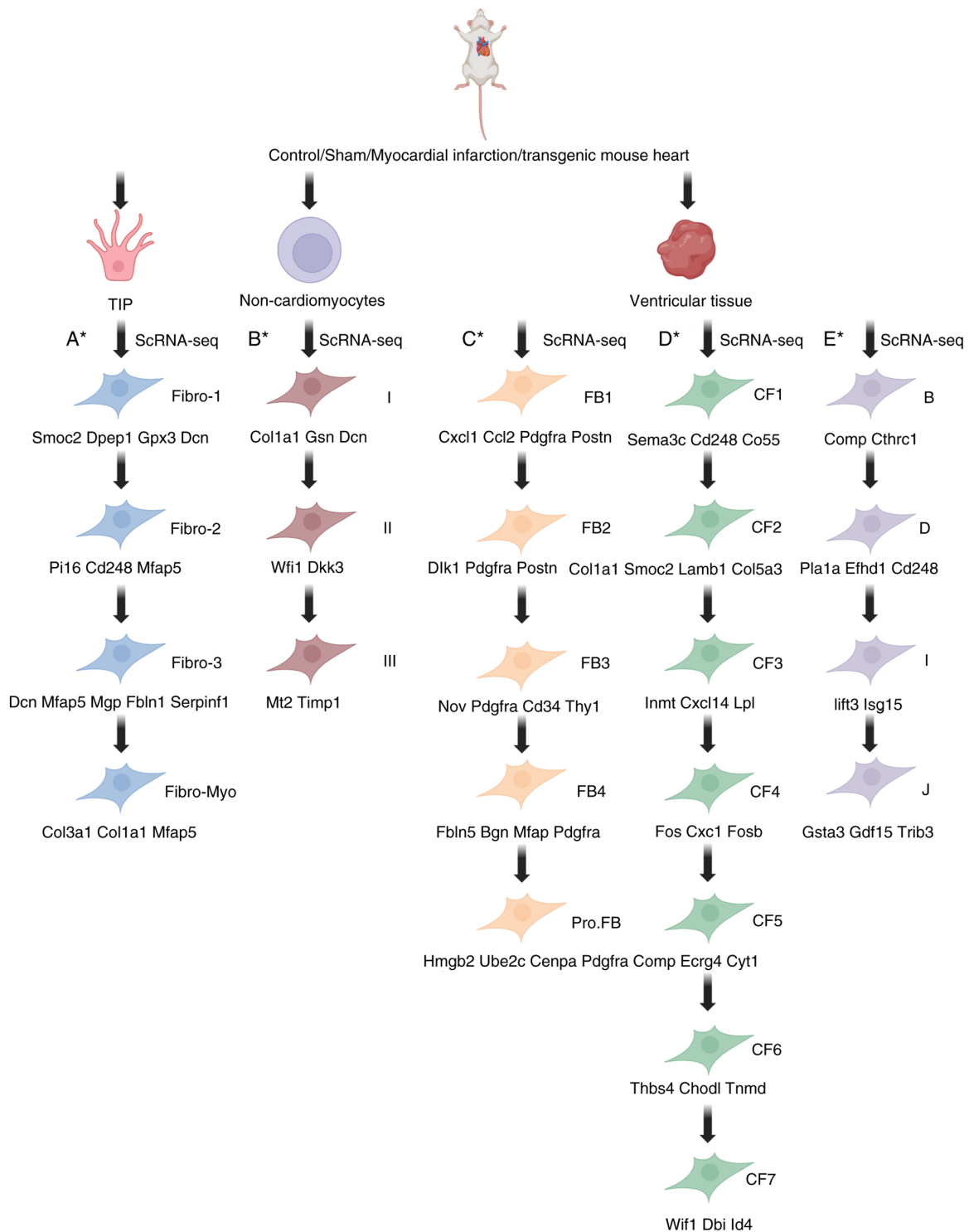


Figure 3. Fibroblast cluster typing and marker genes for each cluster. The high expression of myofibroblast marker genes *Acta2*, *Tagln* and *Cthrc1* in FB1/B group/Fibro-Myo; enrichment of the differentiation inhibitory gene *Dlk1* in FB2; the specific expression of the proliferation gene *Mki67* in Pro.FB; the upregulation of matrix homeostasis genes *Fbln5* and *Bgn* in FB4; the significant expression of sclerosis-related genes *Comp*, *Cilp* and *Angptl7* in CF5/CF6 and the enrichment of osteogenic genes *Adamtsl2* and paracrine factor *SFRP2* in MFCs. This indicates that they are respectively involved in fibrotic driving, differentiation inhibition, proliferation repair, matrix remodeling and chronic scar hardening. (A*) Fibroblast clusters of cardiac interstitial cell origin and marker genes. (B*) Fibroblast clusters of non-cardiomyocyte origin and marker genes. (C*-E*) Fibroblast clusters of cardiac ventricular tissue origin and marker genes. The CF1-CF4 subsets highly express genes related to the degradation of homeostasis fibroblasts (*Hsd11b1*, *Lpl* and *Dpt*) and ECM; CF5 and CF6 express genes related to the activation of fibroblasts, cartilage development and ossification, including *Angptl7*, *Cilp*, *Comp*, *Ecrg4*, *Fmod*, *Postn*, *Meox1* and *Thbs4*. ScRNA-seq, single-cell RNA sequencing; Fibro1, the matrix generates fibroblasts; Fibro2, damage-responsive fibroblasts; Fibro3, precursor fibroblasts; Fibro-my, myofibroblasts (highly expressing *Cthrc1* and *Ddah1*); Type I, quiescent interstitial progenitor cells; Type II, cells responding to transitional state damage; Type III, terminally differentiated profibrotic cells; FB1, stromal homeostasis fibroblasts; FB2, inflammatory injury response fibroblasts (specifically expressing *Dlk1*); FB3, precursor of myofibroblasts (specifically expressing *Nov*, *Thy1*, *Pi16*, *Axl* and *Cd34*); FB4, perivascular repair fibroblasts (highly expressing *Fbln5*, *Bgn* and *Mfap*); Pro.FB, regenerative potential progenitor cells; CF1, resting-state progenitor cells; CF2, immunomodulatory fibroblasts; CF3, stromal homeostasis fibroblasts; CF4, transition state precursor fibroblasts; CF5, contractile myofibroblasts; CF6, perivascular repair fibroblasts; CF7, lipid metabolism-related fibroblasts (responsive growth factor); B, the stromal microenvironment maintains fibroblasts; D, antigen-presenting fibroblasts; I, myofibroblast precursor cells; J, terminal contractile myofibroblasts.

contribution ratio on day 3. Type III fibroblasts were markedly higher on day 3 post-infarction.

Within the Type I fibroblast population, three closely related subpopulations of ZsGreen+ epicardial-derived fibroblasts (EpiDs) were identified: steady-state epicardial-derived fibroblasts (HEpiDs), progenitor-like state fibroblasts (PLS) and late-phase fibroblasts (LR). The HEpiD population highly expressed genes involved in the response to organic substances and metabolic effectors, such as *Dpep1*, *Lpl*, *Hsd11b1* and *Cxcl14*. The PLS population remained relatively stable across all time points, with comparatively high expression of genes related to cell migration and morphogenesis such as *Cd248* and *Pi16*. The LR population, which was predominantly present during the post-infarction maturation phase (days 14–18), showed high expression of genes involved in cellular differentiation, osteogenesis and matrix remodeling, such as *Adamts2*.

Type II fibroblasts specifically expressed genes closely related to valve leaflet development and were involved in endochondral ossification, Wnt signaling and structural morphogenesis. However, Type III fibroblasts have not been fully characterized to date.

Additionally, three main myofibroblast (Myofb) populations were observed after subclustering: Myofb (*Acta2*, *Cthrc1*), proliferative myofibroblasts (*ProlifMyofb*; *Acta2*, *Cthrc1* and cell cycle genes) and a group of stromal fibroblasts (MFCs) similar to those found in mature scars (48). Myofb expressed *Acta2* and *Cthrc1* and their proportion increased markedly on days 3, 5 and 7 post-infarction (peaking at day 5). *ProlifMyofb* expressed *Acta2*, *Cthrc1*, the pro-repair paracrine factors *SFRP2* and *CLU* and cell cycle genes. The proportions increased markedly on days 3 and 5 post-infarction, with a decreasing trend on day 7. Stromal fibroblast-like cells MFCs increased markedly in proportion on days 14–28 post-infarction (mature scar phase). The aforementioned key populations (such as early activated FI/EpiDs, proliferating *ProlifMyofb*, MFCs contributing to bone/chondrocyte-like ECM) and their specific marker genes (*Pi16*, *Adamts2*, *Comp* and *Cilp*) and pathways (Wnt signaling) provide new potential targets for anti-fibrotic and amelioration of scar remodeling therapies.

In 2020, Wang *et al* (39) identified five cardiac fibroblast (FB) subclusters, labeled FB1–FB4 and proliferating fibroblasts (Pro.FB), all of which highly expressed FB marker genes such as *Postn*, *Col1a1* and *Pdgfra*. Specific genes defining each cluster were identified using differential expression analyses (the marker gene expression for each subcluster is shown in Fig. 3C). Partial FB1 cells expressed high levels of myofibroblast marker genes, including *Acta2* and *Tagln*, but low levels of the static FB marker *Pdgfra*, reflecting the early steps in the activation and differentiation of cardiac FBs into myofibroblasts (48,53). FB2 expressed *Dlk1*, an inhibitor of myofibroblast differentiation (54). The specific genes expressed by FB3 may be related to normal fibroblast physiological functions (55–58). High expression of extracellular matrix organization-related genes such as *Fbln5*, *Bgn* and *Mfap* in FB4 may be associated with the regulation of matrix structure and homeostasis and could be considered as a regulatory target for improving scar elasticity. By comparing the number dynamics of FB subclusters between 1- and 8-day-old neonatal mice following MI, it was found that the percentage of Pro.FB was markedly higher in 8-day-old neonatal mice at 3 days post-infarction, indicating

that FB proliferation precedes cardiac fibrosis. This observation aligns with previous findings FB proliferation occurred following MI in 8-day-old neonatal mice (55). FB1 showed the highest increase in P8-injured hearts, demonstrating it as a core fibrotic effector group. By temporally regulating specific subgroups (inhibiting early amplification of Pro.FB and blocking the fibrotic transformation of FB1), the fibrotic process after infarction can be precisely intervened.

In 2023, Tani *et al* (38) classified fibroblasts into seven clusters by performing scRNA-seq on left ventricular tissues from control mice (*Tcf21iCre*/Tomato) and TTg (*Tcf21iCre*/Tomato/*MGTH2A*) mice 3 months following MI. These clusters were labeled CF1 to CF7, with their respective marker genes shown in Fig. 3D. CF5 and CF6 numbers increased in Ctrl-MI and decreased in TTg-MI mice compared with controls. Clusters CF1–CF4 expressed genes associated with steady-state fibroblasts (*Hsd11b1*, *Lpl* and *Dpt*) and ECM degradation, while CF5 and CF6 expressed genes related to activated fibroblasts, chondrogenesis and ossification, including *Angptl7*, *Cilp*, *Comp*, *Ecrg4*, *Fmod*, *Postn*, *Meox1* and *Thbs4*. CF7 expressed genes responsive to growth factors. Future studies may reveal a direct association between CF5/CF6 and its genetic signature (*Comp*^{+/*Cilp*⁺/*Postn*⁺) and elevated cardiac stiffness in advanced stages. *Angptl7*, *Thbs4* or downstream signaling pathways could be used as targets for the development of novel antifibrotic drugs. CF5/CF6 signature profiles or expression levels could be used as non-invasive indicators (serum COMP assay) to assess the severity of chronic fibrosis and response to therapy. Non-invasive indicators include serum COMP and CILP test. Patients with high CF5/CF6 ratios may require targeted antisclerotic therapy.}

Ruiz-Villalba *et al* (52) performed single-cell sequencing on cardiac tissues from healthy and post-infarction *Col1a1*-GFP mice at 7 and 14 days post-infarction, identifying 10 fibroblast clusters (A–J). Clusters B, D, I and J showed distinct expression profiles, as shown in Fig. 3E. Clusters F and H–K remained unchanged across different time points. Cluster I decreased to a minimum and then increased at 14 dpi, while the percentage of cluster B markedly increased following MI, peaking at day 14 and then declining. Cluster B was markedly enriched in the infarct zone at 7 days post-MI, decreasing by day 30 (52). Cluster B had pathways and gene ontologies related to ECM organization, cell proliferation and cell-matrix adhesion (56,57). The top marker of subcluster B, *Cthrc1*, has been associated with vascular remodeling and fibrosis (58–61). The highly specific expression of ECM-related genes in subcluster B suggests a strong association with fibrotic scar formation, which exhibits intense necrotic features after infarction and localizes to damaged tissue. Precision targeted intervention of *Cthrc1* or its downstream pathway inhibits cluster B activation and blocks early fibrotic outbreak. The development of relevant assays targeting serum profiles of genes characteristic of group B (*Cthrc1*) to assess fibrosis activity and therapeutic efficacy following MI should be considered.

In 2022, Janbandhu *et al* (62) performed scRNA-seq on tdTomato+ CD31⁺CD45⁺ fibroblasts from male magnetic mice 8–12 weeks after a sham operation or MI. The fibroblasts were classified into eight clusters: F-SL, F-SH, F-Trans, F-WntX, F-Act, F-CI, F-Cyc and F-IFNS (IF Stimulated). The F-Act cluster was markedly expanded following MI;

Table IV. A typical study on ScRNA-seq of monocyte subsets.

Source of organization	Cardiac interstitial cell populations in mice	Ventricular tissue in mice	Myocardial CD45 ⁺ leukocytes in mice	Non-cardiomyocytes from mice	CD45 ⁺ non-cardiomyocytes from mice
Methods	10X Genomics ScRNA-seq	10X Genomics ScRNA-seq	10X Genomics ScRNA-seq	ScRNA-seq	ScRNA-seq
Number of cells captured	13,331 cells	17,320 cells	30,135 cells	27,349 cells	26,275 cells
Clusters	M1MO, M1MΦ, M2MΦ, MAC-IFNIC, MAC-TR, MAC8, MAC7, MAC6	M1, M2	MAC-TRc1-3, Mo-Ly6c, Mo-Ear2, MAC-Olr1, MAC-Gpnmb, MAC-Lars2, IFNIC	IFNIC, MAC-Mo/M1, MAC-M2, MAC-TR, MAC-3, MAC-APC, MAC-4, MAC-Fib, MAC-Endo	MΦ1, MΦ2, MΦ3

Data from Nona *et al* (43), Wang *et al* (39), Zhuang *et al* (63), Zhuang *et al* (51) and Xu *et al* (67) (all from the 2019-2023 literature specifically analyzing cardiac macrophages by ScRNA-seq and with impact factors fluctuating from a score of 2.9-6.9, all of which identified different macrophage clusters and explored associated functions and the experiments were conducted using publicly available datasets). ScRNA-seq, single-cell transcriptome sequencing; MΦ, macrophages; MO/Mo, monocytes; MAC, macrophage; TR, tissue resident; IFNIC, IFN inducible cell; MAC-Endo, endothelial-like macrophages; MAC-Fib, FB-like macrophages; MAC-APC, macrophage-antigen presenting cell.

the F-CI cluster was markedly increased in cKO (Hif-1a Δ flox/-;PdgfraMCM⁺) hearts following MI; and the number of F-SLs was markedly increased in cKO and HET (Hif1a flox/+; PdgfraMCM⁺) hearts following MI. The F-Act cluster specifically exhibited downregulated expression of genes encoding negative regulators of cell signaling, particularly genes involved in key pathways regulating CF proliferation such as MAPK-ERK1/2 and fibroblast growth factor (FGF). This suggests that hypoxia-inducible factor 1 (HIF-1) α deletion may deregulate the inhibition of the proliferative pathway and promote the conversion of F-Act to the proliferative state (F-CI). The proliferation-associated subgroups F-CI (proliferative intermediate state) and F-Cyc (proliferative state) also showed downregulation of HIF-1 target genes, directly confirming the central role of HIF-1 α signaling in the regulation of CF proliferative program. The HIF-1 α pathway and its downstream regulation of key subgroups (F-Act, F-CI) and signaling nodes (MAPK-ERK, FGF pathway negative regulatory factors) are potential targets for intervention. Modulating HIF-1 α activity or interfering with its downstream effector molecules may precisely control pathological CF proliferation and activation and attenuate deleterious fibrosis. The identified specific subgroups (markedly expanded F-Act, accumulated F-CI and F-SL in cKO) and their characteristic gene expression profiles are expected to serve as novel molecular markers to assess the development of fibrosis.

Monocyte/macrophage heterogeneity and associated changes following MI. Macrophages are key players in inflammatory response and post-infarction cardiac remodeling. The literature detailing their detection via single-cell sequencing is summarized in Table IV. In 2019, Farbehi *et al* (43) classified monocytes and macrophages into several clusters through single-cell RNA-seq, as follows: M1MO, M1MΦ, M2MΦ, MAC-IFNIC, MAC-TR, MAC8, MAC7 and MAC6. The M1MΦ cluster was identified as a derivative of classical monocytes and was characterized by Ccr2^{high}Adgre1 (F4/80)⁺ Ly6c2⁺ H2-Aa (MHC-II)⁺, driving the early inflammatory response. Non-classical M2MΦ cells, which were characterized by Ccr2^{high}Adgre1 (F4/80)⁺ H2-Aa (MHC-II) high-Ly6c2, participate in repair through the non-arginase pathway (Arg1^{low}), providing a novel immunomodulatory mechanism. After MI, the M1MΦ and M1MO populations increased at 3 dpi, while the M2MΦ population expanded at 7 dpi. On the third day following MI, targeted inhibition of M1MΦ (such as anti-CCR2 antibody) was carried out to alleviate inflammatory damage; on the 7th day following MI, the M2MΦ/Cx3cr1⁺ population (such as Cx3cr1 agonists) was enhanced to promote tissue repair.

In 2020, Wang *et al* (39) used Adgre1 as a specific marker for mononuclear macrophages and classified macrophages into M1 and M2 clusters through higher-resolution sub-clustering analyses (the marker genes for these clusters are shown in Fig. 4A). An increase in macrophage and monocyte

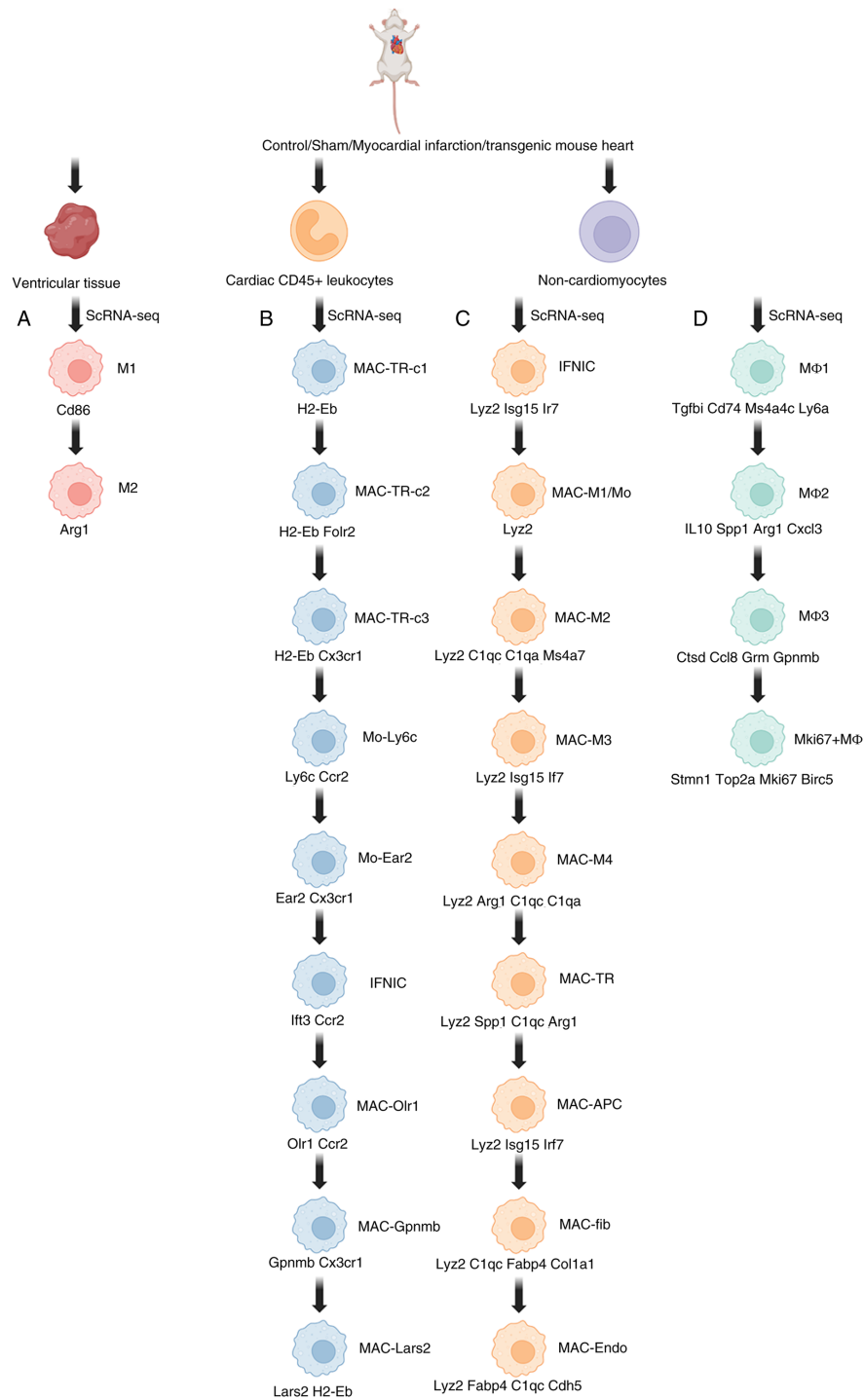


Figure 4. Monocyte macrophage clusters typing and marker genes for each cluster. High expression of pro-inflammatory genes *Ly6c2*, *S100a8* and the *C1q* family (**C1qa/b/c**) in M1/MAC-Mo; enrichment of repair genes *Spp1*, *Cd163* and *Mrc1* in M2/MAC-TR; the significant upregulation of anti-fibrotic genes *Fabp5* and *Gpnmb* in *Bhlhe41*+MΦ; the activation of phagocyte-related gene *Fcrl2* in MAC-Gpnmb; and the co-expression of the cross-lineage genes *Col1a1* (fibroblast) and *Cdh5* (endothelial) in MAC-Fib/MAC-Endo indicates that they are respectively involved in inflammation initiation, tissue repair, fibrosis inhibition, fragment clearance and lineage plasticity. (A) Monocyte cell clusters of Cardiac ventricular tissue origin and marker genes. (B) Monocyte cell clusters of Cardiac CD45⁺ leukocytes origin and marker genes. (C) Monocyte cell clusters of non-cardiomyocyte origin and marker genes. (D) Monocyte cell clusters of non-cardiomyocyte (temporary cardiac resident macrophage subset) origin and marker genes. The *Ccr2*_{hi} cluster contains subgroups such as MAC-TR, MAC-Fib and MAC-Endo and is characterized by highly expressing *Lyve1* and *Cx3cr1*. ScRNA-seq, single-cell RNA sequencing; M1, pro-inflammatory/damage-responsive macrophages (highly expressing *C1qa*, *C1qb*, *C1qc*, *Pf4*); M2, repair/regeneration-promoting macrophages (highly expressing *C1qa*, *C1qb*, *C1qc*, *Spp1*); MAC-TR-c1, resting-state tissue-resident macrophages; MAC-TR-c2, damage-responsive macrophages; MAC-TR-c3, ECM remodeling macrophages; Mo-Ly6c, inflammatory mononuclear derived monocytes; Mo-Ear2, anti-inflammatory readiness monocytes; IFNIC, interferon-responsive macrophages; MAC-Olr1, lipid phagocytic macrophages; MAC-Gpnmb, profibrotic macrophages; MAC-Lars2, metabolic repair macrophages; IFNIC, interferon-responsive macrophages; MAC-Mo/M1, classic pro-inflammatory macrophages; MAC-M2, repair macrophages; MAC-TR, tissue-resident macrophages; MAC-3, lipid-clearing macrophages; MAC-APC, antigen-presenting macrophages; MAC-4, profibrotic macrophages; MAC-Fib, profibrotic macrophages; MAC-Endo, promotes angiogenesis macrophages; MΦ1, homeostatic tissue-resident macrophages (expressing *Timd4*⁺ and *Lyve1*); MΦ2, repair hub macrophages (expressing *Bhlhe41*⁺, *Vegfa*⁺); MΦ3, profibrotic macrophages (expressing *Spp1*⁺ and *Mmp9*⁺); Mki67⁺MΦ, proliferative and migratory macrophages (expressing *Mki67*⁺ and *Cxcr4*⁺).

percentages was observed at all analyzed time points post-MI. However, the timing and magnitude of macrophage elevation differed depending on the neonatal stage, suggesting that macrophages may play a role in post-infarction cardiac remodeling and myocardial regeneration. Hearts from P1 mice exhibited higher percentages of macrophages and monocytes 1 dpi, while hearts from P8 showed elevated macrophage and monocyte percentages at 3 dpi. At 1 dpi, P1 mice demonstrated a rapid increase in the M1 and M2 macrophage populations compared with controls, with a unique level of M2 infiltration not observed at other time points. Additionally, both P1 and P8 neonatal mice showed a pronounced increase in M1 and M2 macrophage composition at 3 dpi compared with controls (39). M2 macrophages secrete anti-inflammatory cytokines, promoting wound healing and tissue repair, which may support cardiac regeneration in neonatal mice and it provides a theoretical basis for promoting cardiac repair via enhancing the polarization or function of the M2 phenotype. Furthermore, the macrophage-secreted factor CLCF1 was found to enhance cardiomyocyte proliferation (39), which may provide options for regenerative therapy.

In 2022, Zhuang *et al* (63) identified nine subclusters of mononuclear macrophages through single-cell RNA-seq: 3 intratissue macrophage clusters (MAC-TRc1-3), 2 monocyte clusters (Mo-Ly6c and Mo-Ear2) and 4 ischemia-associated macrophage subclusters with differentially expressed marker genes (MAC-Olr1, MAC-Gpnmb, MAC-Lars2 and IFNIC), with specific marker genes detailed in Fig. 4B. MAC-Olr1 numbers increased at 1 dpi and were characterized by the upregulation of SASP and glycolysis pathways. The MAC-Gpnmb subcluster markedly increased at 7 dpi and showed a powerful devouring ability (it was positively correlated with phagocytosis and cellular FAO levels). The Mo-Ly6c cluster was related to pro-inflammatory responses and angiogenesis. The Mo-Ear2 cluster may be involved in adaptive immune regulation following MI, providing a theoretical basis for precisely regulating the differentiation of monocytes into beneficial phenotypes (such as inhibiting pro-inflammatory Mo-Ly6c and enhancing regulatory Mo-Ear2), which is helpful for balancing inflammation and repair. Among the three MAC-TRs clusters, MAC-TR-c2 expresses *Folr2* and hematopoietic cell lineages, lysosomes and endocytic signals are enriched in *Folr2*⁺ macrophages, making it an ideal target for promoting cardiac repair. The proportion of *Folr2*⁺ macrophages decreased on the first day following MI and recovered 7 days following MI.

Zhuang *et al* (51) identified seven macrophage clusters: IFNIC, MAC-Mo/M1, MAC-M2, MAC-TR, MAC-3, MAC-APC and MAC-4. The marker genes for the newly identified clusters are detailed in Fig. 4C. The proportions of MAC-Mo/M1 and MAC-M2 increased at 3 dpi, with a particularly rapid rise in MAC-M2, while MAC-TR showed greater proliferation by 7 dpi (51). Pseudotemporal analysis of macrophage populations revealed the presence of fibroblast-like macrophages (MAC-Fib) and endothelial-like macrophages (MAC-Endo), which expressed fibroblast markers (*Colla1*) and endothelial markers (*Cdh5*), respectively. Tissue-resident macrophages (MAC-TR) showed increased expression of *Cx3cr1* and *H2-Aa*, while downregulating the pro-inflammatory gene *Ly6c2*. Some of these cells also expressed *Cd163* and

Mrc1 while lacking *H2-Aa*, suggesting an anti-inflammatory role in ischemic injury.

Further exploration of the core transcriptional regulation driving macrophage differentiation was conducted using single-cell regulatory network inference and clustering analysis (64). A total of two clusters were identified: The *Ccr2_hi* cluster contains subgroups, including MAC-Mo/M1 and IFNIC; is enriched in pro-inflammatory transcription factors such as *Stat1* and *Irf7*; and it was positively associated with myeloid cell activation, immune response and extracellular secretion pathways (51). Previous research has found that excessive activation of *Irf3* is associated with excessive interferon production and adverse inflammatory responses (65). The *Ccr2_lo* cluster contains subgroups such as MAC-TR, MAC-Fib and MAC-Endo and is characterized by highly expressing *Lyve1* and *Cx3cr1* (66). This cluster is markedly enriched in the gene set involved in the regulation of cell proliferation and the response to growth factor HGF/TGF- β and is regulated by transcription factors such as *Egr1* and *Jund* (51). Functional comparative analysis clearly supported the *Ccr2_lo* cluster (especially MAC-TR) to exert protective effects of self-renewal and anti-inflammation following MI (51). Enhancing the anti-inflammatory function of MAC-TR and the angiogenesis ability of MAC-Endo by using small molecule agonists is a direction for treating cardiac remodeling following MI. In addition, *in vitro* expansion of *Ccr2_lo* characteristic cells and transplantation into the infarcted area is also a means to achieve anti-inflammatory and vascular regeneration simultaneously.

In 2023, Xu *et al* (67) identified two major tissue macrophage populations at steady state (Sham) using scRNA-seq in 8-10 week-old male C57BL/6J mice: *LYVE1*⁺*MHCII*⁺*CCR2*⁻ and *LYVE1*⁺*MHCII*⁺*CCR2*⁺. The two populations were markedly reduced at 3 days post-MI. Four additional macrophage clusters showed a significant increase in response to MI: *MΦ1*, *MΦ2*, *MΦ3* (referred to as *Bhlhe41*⁺*MΦ*) and *Mki67*⁺*MΦ*. *MΦ3*, which exhibited higher *Bhlhe41* activation. The specific marker genes for each cluster are detailed in Fig. 4D. The number of *MΦ3* cells markedly increased on day 3 post-MI, peaked on 7 dpi and returned to baseline by 14 dpi, clearly indicating the key intervention time window targeting this repair macrophage. In addition, *Fabp5*, *Gpnmb* and *Ccl8* were markedly upregulated in *MΦ3* at 3 dpi, whereas *Il1b*, *Il10*, *Tgfb1* and *Nfkb1* were downregulated. Cell death-related markers, including those for autophagy, apoptosis and necroptosis, were also downregulated, along with O-GlcNAc modification, ubiquitylation levels and hypoxic injury markers in *Bhlhe41*⁺ *MΦ* cells. These macrophages played roles in collagen catabolic processes, positive regulation of tissue remodeling and lipid metabolism pathways. Notably, *Bhlhe41*⁺ *MΦs* were the only macrophage cluster enriched in cardiac fibroblast lineage cells (*Pdgfr-GFP*⁺). It is suggested that this subgroup plays a core protective role in restricting the dilation of the infarcted area and preventing pathological fibrosis by inhibiting the activation of myofibroblasts and excessive collagen deposition. The *Bhlhe41*⁺ m4 subgroup demonstrates a strong repair ability. In the future, therapeutic strategies can be focused on promoting its expansion and recruitment, maintaining its phenotype and function and simulating its secretory factors/functions, etc.

Table V. A typical study on ScRNA-seq of T/B cell subsets.

Source of organization	Cardiac interstitial cell populations in mice	Non-cardiomyocytes from mice	Cardiac and mediastinal lymph node B cells in mice	Myocardial CD45 ⁺ leukocytes in mice
Methods	10X Genomics ScRNA-seq	ScRNA-seq	10X Genomics ScRNA-seq, S2 Cartridge ScRNA-seq	10X Genomics ScRNA-seq
Number of cells captured	13,331 cells	27,349 cells	6,588 cells	30,135 cells
Clusters	TC1-Cd8 (Cd8a ⁺), TC2Cd4 (Cd4 ⁺ Lef1 ⁺)	Naïve T-cell, effector T-cell, regulatory T-cell, NK-cell	B1, B2, MZ, GC, hB, CD74 ⁺ cluster, IFNR, Cyc	CD4-C1-CCR7 and CD8-C1-CCR7 represent naïve T cells and CD4-C2-CXCR3 and CD8-C2-Gzmk represent effector T cells; B cell-Cd20, B cell-Cd23

Data from Farbehi *et al* (43), Zhuang *et al* (51), Heinrichs *et al* (71) and Zhuang *et al* (63) (all from the 2019-2022 literature specifically analyzing cardiac T/B cells by single-cell sequencing and with impact factors fluctuating from a score of 2.9-6.4, all of which identified different T/B cell clusters and explored associated functions and the experiments were conducted using publicly available datasets). ScRNA-seq, single-cell transcriptome sequencing; TC, T-cell; MZ, marginal zone B-cells; GC, germinal center B-cells; hB, heart-associated B-cells; IFNR, an IFN-responsive transcript-rich subpopulation; Cyc, a cluster enriched in cell cycle-related and ribosome biogenesis transcripts.

T-cell and B-cell heterogeneity and associated changes following MI. T-cells and B-cells are critical participants in the post-infarction inflammatory response. The literature on their role, as identified by single-cell sequencing, is summarized in Table V. In 2019, Farbehi *et al* (43) identified two T-cell clusters through single-cell RNA-seq: TC1-Cd8 (Cd8a⁺), representing cytotoxic T-cells and TC2-Cd4 (Cd4⁺ Lef1⁺), which likely represents helper T-cells.

In 2020, Zhuang *et al* (51) performed single-cell sequencing on non-cardiomyocytes from 8-10 week-old male mice, comparing sham-operated mice and those at 3, 7 and 10 days post-infarction. The authors classified T-cells into four clusters: Naïve, effector, regulatory and NK cells. The marker genes for each cluster are detailed in Fig. 5A. A significant infiltration of T-cells was observed 7 days following MI. The effector T-cell population showed upregulation of Ccl6 and Il1b and was enriched for pathways associated with cell activation and immune system regulation. The regulatory T-cell population positively associated with inflammatory pathways of humoral immune responses and myeloid leukocyte differentiation. Effector T-cells were enriched for pro-inflammatory regulators such as Irf5 and Fosb, while regulatory T-cells were enriched for activated Sp1 regulators. Exploring drugs targeting transcriptional regulatory factors such as Irf5 or Fosb

can regulate the activation and function of effector T cells. Regulating the immunomodulatory properties and Sp1-related stability exhibited by T cell subsets is a potential therapeutic strategy for promoting immune tolerance and improving the cardiac repair environment.

In 2022, Zhuang *et al* (63) conducted scRNA-seq of live myocardial CD45⁺ leukocytes. Samples were collected at 7 days post-sham surgery and at 1 or 7 dpi. The authors identified CD4-C1-CCR7 and CD8-C1-CCR7 as markers for naïve T-cells, while CD4-C2-CXCR3 and CD8-C2-Gzmk were markers for effector T-cells. These markers were positively associated with the differentiation of Th17, Th1 and Th2 cells, as well as the PD-L1 signaling pathway. CD4-C2-CXCR3 and CD8-C2-Gzmk T-cell populations both markedly increased at 7 dpi. Cxcr3⁺ CD4 T-cells and Gzmk⁺ CD8 T-cells activated key adaptive immune responses via Fos and Fosb activity on 7 dpi. Targeting specific effector T cell subsets or their activation pathways (such as Fos/Fosb) may serve as a new strategy for regulating cardiac inflammation and repair following MI.

In 2022, Qian *et al* (68) observed that CD8⁺ effector T-cells secreted effector molecules such as GZMB, GNLY and PRF1, which promoted apoptosis and EC shedding, leading to plaque erosion. This was demonstrated via scRNA-seq of peripheral blood mononuclear cells from 10 patients with AMI (5 with

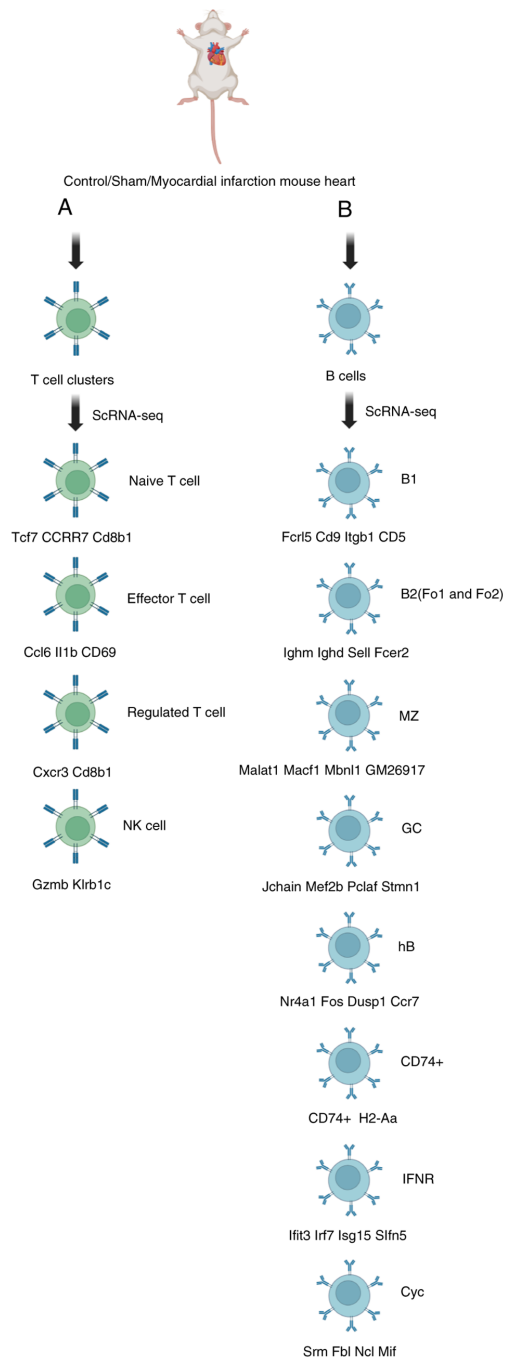


Figure 5. T and B cell clusters typing and marker genes for each cluster [MZ marker genes related literature]. High expression of pro-inflammatory factor IL-1 β , chemokine Ccl6 and transcription factor Irf5/Fosb in effector T cells regulate the enrichment of immune regulatory pathways (such as Sp1) in T cells; co-activation of the dual-chemokine receptor Cxcr5/Ccr7 and the tissue repair factor Tgfb1 in hB cells; and upregulation of the cell cycle gene Trp53/Cdc27 in Cyc B cells indicates that they are respectively involved in inflammatory drive, immunosuppression, repair chemotaxis and proliferation responses. (A) T cell clusters typing and marker genes for each cluster. (B) Cell clusters typing and marker genes for each cluster. ScRNA-seq, single-cell RNA sequencing; Naive T cells, immune response preparatory T cells; effector T cells, pro-inflammatory injury type T cells (expressing Ccl6 and IL-1 β); regulatory T cells, immunosuppressive T cells (rich in Sp1 regulators); NK cells, direct killer T cells; B1 cells, natural immune barrier type B cells; B2 cells, lymphoid tissue localization type B cells; marginal zone B cells (MZ), rapid antibody-responsive B cells; germinal center B cells (GC), antibody-affinity mature B cells; hB, heart-associated B cells, repair of core-type B cells (expressing Tgfb1, Cd69, Cxcr5 and Ccr7); CD74 $^{+}$, antigen-presenting type B fine (expressing Cd74 $^{+}$); IFNR cells, interferon-responsive B cells; Cyc cells, proliferation type B cells (expressing Trp53, Cdc27, Mrto4, Nhp2, Ranbp1 and Ncl Gnl3).

plaque rupture and 5 without) (69). Upregulation of CXCR4, B4GALT1 and TNFAIP3 was associated with angiogenesis and wound healing, thereby promoting plaque healing (70). It may help evaluate the plaque stability or healing potential of patients with AMI and provide a basis for individualized intervention.

In 2021, Heinrichs *et al* (71) performed single-cell RNA and B-cell receptor sequencing on 20 B-cells purified from the heart and mediastinal lymph nodes on day 5 post-MI, revealing extensive phenotypic diversity among B-cells infiltrating the infarcted heart. scRNA-seq of 6,588 B-cells using UMAP revealed nine B-cell subpopulations in the heart, including B1, B2 (including follicular B-cells), marginal zone B-cells (MZ), germinal center B-cells (GC) and heart-associated B-cells (hB). Additionally, three smaller clusters were identified: a CD74 $^{+}$ cluster, an IFN-responsive transcript-rich subpopulation and a cluster enriched in cell cycle-related and ribosome biogenesis transcripts (Trp53, Cdc27, Mrto4, Nhp2, Ranbp1, Ncl and Gnl3). This last cluster likely represents cycling B-cell subpopulations (Fig. 5B).

With the exception of the hB population, all other clusters underwent significant numerical expansion post-MI. B cells are the only subpopulation expressing Cxcr5. The hB cell population upregulates the expression of transforming growth factor β 1 (Tgfb1) and the activation marker Cd69, suggesting that it is in an activated state and may be involved in the process of immune regulation or tissue repair. It is also the only subpopulation that simultaneously highly expressed both Cxcr5 and Ccr7 (double positive). Flow cytometry confirmed that CCR7 $^{+}$ CXCR5 $^{+}$ B cells were specifically enriched in the infarcted scar tissue. Their numbers peaked at 7 dpi (71). The pro-repair properties of hB cells (such as secreting Tgfb1) and their specific localization in scar tissue make them potential therapeutic targets for promoting myocardial repair or inhibiting adverse remodeling. Regulating its chemotaxis (such as the CXCR5/CCR7 pathway) or functions (such as the Tgfb1 signaling) may have therapeutic value. The expansion of different B cell subsets (such as pro-inflammatory Cyc B that may regulate/repair hB) reflects the complex immune response following MI, providing ideas for developing the regulation of immune balance and optimizing the repair process.

In 2022, Zhuang *et al* (63) identified two B-cell clusters with differential expression of Cd20 (Ms4a1) and Cd23 (Fcer1g): B-cell-Cd20 and B-cell-Cd23. B-cell-Cd20 showed a slight expansion at 1 dpi, while B-cell-Cd23 expanded at 7 dpi. B cell-CD20 highly expressed CD20 (regulating B cell maturation, differentiation and BCR signal transduction) and CD69 (an early activation marker). B cell-Cd23 specifically and highly expresses CD23 (a low-affinity IgE receptor involved in the activation regulation of B cells). The CD20 $^{+}$ or CD23 $^{+}$ B cell subsets can be used as markers for evaluating the immune response following MI.

5. Interactions between cells in the heart following MI

Increased intercellular communication is a hallmark of cardiac repair and plays a critical role in cardiac remodeling (72). The analysis of cellular interactions through single-cell sequencing has deepened the understanding of post-infarction cellular interactions, as demonstrated in the following studies.

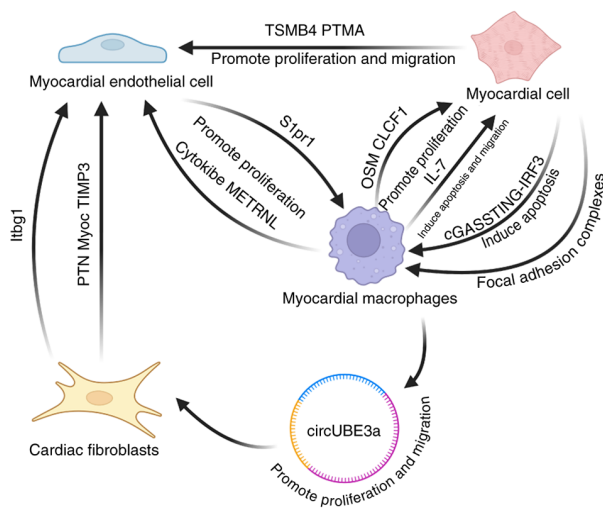


Figure 6. Major Interactions between cells in the heart following MI. It mainly demonstrates the interactions among cardiomyocytes, macrophages, endothelial cells and fibroblasts in the heart after myocardial infarction and also involves a few epicardial cells. It clearly shows the roles of cardiomyocytes in stress compensation after myocardial infarction, endothelial cells in promoting angiogenesis and regulating inflammation, macrophages in promoting inflammation, angiogenesis and remodeling and fibroblasts in repairing scar formation and promoting fibrosis. OSM, oncostatin M; CM, cardiomyocyte; MMP14/MT1-MMP, membrane-type matrix metalloproteinase-1; METRNL, meteorin-like.

Cardiomyocytes and macrophages. In 2020, Li *et al* (73) found that macrophage secretion of oncostatin M (OSM) promoted cardiomyocyte (CM) proliferation and cardiac regeneration after infarction. Wang *et al* (39) observed that mononuclear macrophages secreted CLCF1, which promoted neonatal CM proliferation. Yan *et al* (74) in 2021 showed that post-infarction macrophages could induce CM apoptosis and enhance CM migration *in vitro* via IL-7. Wong *et al* (75) concluded that post-infarction CCR2⁺ macrophages interact with CMs via adhesion plaque complex markers such as β -integrins. Hu *et al* (76) demonstrated that, after infarction, CMs that had undergone death could interact with macrophages through the cGAS-STING-IRF3 pathway, potentially inducing apoptosis in healthy CMs.

Cardiomyocytes and ECs. In 2021, Gladka *et al* (77) found that ZEB2 induced cardiomyocytes to produce TMSB4 and PTMA, which drove EC migration and proliferation, thereby promoting angiogenesis and improving cardiac function.

Macrophages and ECs. In 2021, Kuang *et al* (78) demonstrated that ECs interacted with macrophages in a contact-dependent manner via the S1P/S1PR1/ERK/CSF1 signaling pathway, promoting the proliferation of anti-inflammatory macrophages in damaged cardiac tissues and reducing adverse cardiac remodeling post-MI. Alonso-Herranz *et al* (79) found that post-MI cardiac macrophages increased MMP14 (MT1-MMP) expression, activated TGF- β 1 and triggered paracrine SMAD2-mediated signaling in ECs. Reboll *et al* (80) reported that macrophages interact with ECs via the cytokine metrnL.

Fibroblasts and ECs. In 2019, Farbehi *et al* (43) revealed that myofibroblasts interacted closely with ECs through an

interaction network. Fibroblast populations (F-SH, F-SL, F-Act and F-WntX) frequently interacted with ECs and PDGFRA-GFP⁺ fibroblasts and CD31⁺ ECs showed close spatial associations or direct contact, with F-WntX upregulating ligands such as PTN, MyoC and TIMP3. In 2020, Zhuang *et al* (51) utilized a selected collection of human ligand-receptor pairs (81) and the STRING database (82) to demonstrate that Fibro-Myo and Endo_1 interacted via Itgb1.

Fibroblasts and macrophages. In 2021, Wang *et al* (83) identified that post-infarction macrophages release endosomal membrane-derived vesicles enriched with circUbe3a, which promotes fibroblast proliferation, migration and phenotypic transformation, potentially exacerbating myocardial fibrosis post-MI. Wang *et al* (39) identified RSPO1 as a mediator of cellular crosstalk between epicardial cells and ECs, promoting the angiogenic capacity of ECs during the regeneration of post-infarction neonatal hearts.

In conclusion, interactions between cardiomyocytes and non-cardiomyocytes, as well as between non-cardiomyocyte populations, play an essential role in post-infarction cardiac remodeling. These interactions are summarized in Fig. 6.

6. Summary and prospects

Through single-cell sequencing technology, mainly scRNA-seq and snRNA-seq, different subsets of cardiomyocytes and changes in the proportion of subsets and the expression of different genes following MI have been explored. It has been reported that cardiomyocytes following MI have renewability, which is maintained within 1 week after birth. The aforementioned changes of interstitial cardiomyocytes indicate their different functions. Different interactions occur between cardiomyocytes and stromal cells through regulatory factors, also demonstrating the response of cardiomyocytes and cardiomyocyte stromal cells to MI. Single-cell sequencing can help researchers precisely distinguish the specific roles and changes of different cell subsets in these processes, analyze the signaling pathways and interaction networks of different cell subsets and identify the key molecules and pathways that affect cardiac remodeling, thereby providing more precise targets for treatment following MI.

However, the differences in the time points selected across studies may affect the interpretation and conclusion: Sparse time point designs (such as analyzing only 3 and 28 days) may miss key transitions. The peak of inflammation in mouse models is within 72 h, while in humans it lasts several weeks, resulting in limited functional studies on repair phase cell subsets (such as VEC2 ECs). Technical method differences (such as the sensitivity of 10X Genomics and Smart-seq2) can affect the capture of rare subgroups (such as VEC3). To reduce bias, studies need to adopt a dense time series design, combine spatial transcriptome verification and correct the cross-species time axis to comprehensively analyze the spatio-temporal specific functions of cell subsets. In addition, single-cell sequencing can reveal significant differences and provide a complementary value in cell responses between animal models and clinical samples. Regarding temporal dynamics differences, animal models (such as mouse models) can help analyze h-level cell transformation after injury (such

as Bhlhe41⁺ macrophages expanding at 72 h to mediate repair), while clinical samples (human autopsies/transplanted hearts) only provide single-point snapshots and are difficult to employ for capturing dynamic processes. In animal models, the mononuclear-macrophage transformation is predominant, while in clinical samples, B/T cells are more involved (for example, CXCR5⁺hB cells secrete IL-10 to promote repair). In animal models, there is regeneration in the heart, but in adult human hearts, there is almost no regeneration. The fibroblast subsets in the scar area are more complex (such as the enrichment of the FAP⁺/DDR2⁺ pro-fibrotic subsets).

Future research should more deeply identify and functionally verify the key rare cell subpopulations involved in repair (such as angiogenesis, anti-inflammation and the transition from pro-fibrotic to anti-fibrotic) and regeneration. The combination of scRNA-seq with lineage tracing, CRISPR screening and space technology may help precisely reveal the specific roles of these cells in dynamic changes, fate determination and intercellular communication after injury. Design individualized targeted therapeutic strategies (such as specific antibodies, small interfering RNA and small molecule inhibitors) should be employed for analyzing key dysregulated pathways or harmful cell populations in specific patients. The integration of scRNA-seq and spatial transcriptomics is the core direction in the future, as it can not only identify cell types, but also accurately reveal their spatial distribution, neighborhood associations and local ecological niche characteristics in the infarct, boundary and distal areas. Combining space technology and stromal omics, studying how different cells sense, reshape and respond to regional-specific extracellular matrix changes, is at the core of fibrosis regulation. Inferring cell interactions (such as CellChat and NicheNet) using scRNA-seq data will depict the signal networks that change dynamically following MI in greater depth. In the future, it will be necessary to use secretomics to verify and quantify the activities of key signaling pathways. Deeply integrating multimodal data, utilizing artificial intelligence to mine hidden patterns in complex data, establishing a comprehensive human heart cell atlas (health and disease status), developing minimally invasive biomarkers and intelligent targeted delivery systems and verifying single-cell guided strategies in clinical trials should be the focus of future studies. scRNA-seq is expected to markedly change the current understanding and management of MI within the next decade, moving from the traditional 'one-size-fits-all' treatment to a new era of precise diagnosis and targeted intervention based on individual cellular and molecular characteristics.

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Availability of data and materials

Not applicable.

Authors' contributions

XB, HG, CG, NL, LG and XC contributed to the study conception and design. The first draft of the manuscript was written by XB. Writing was supervised and guided by XC and LG. Data authentication is not applicable. All authors commented on previous versions of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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