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Review

Evolving roles of cardiac fibroblasts in cardiogenesis and immunology, electrophysiology, and aging

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Cardiac fibroblasts (CFs), one of the major groups of cardiac cells, play a prominent role in the cardiac microenvironment through communicating with other cells such as cardiomyocytes, endothelial cells and immune cells. These communications are required not only during cardiac development but also during pathogenesis. CFs are also involved in developmental changes in the post-natal and pre-natal heart through depositing extra cellular matrix (ECM) and maintaining cardiac tissue structure. Furthermore, CFs show both detrimental and beneficial effects in maintenance of the electrophysiology of the heart. Senescent CFs in the cardiac microenvironment influence other cardiac cells through paracrine signaling, which would worsen or cure the diseases. Therefore, there is a need of exclusive study on CFs' role in the developmental stage of the heart, electrophysiology, and senescence. This review discusses the current research about CFs' function, especially the CFs' role in cardiac development, electrophysiology, and senescence, and proposes a certain gap filling future prospective.

Keywords

Cardiac fibroblasts; Cardiac microenvironment; Cardiac development; Electrophysiology; Senescence

1. Introduction

Cardiac fibroblasts (CFs) are mesenchymal in nature. CFs originating from the pro-epicardial organ through epithelial to mesenchymal transition (EMT) are referred to as epicardial fibroblasts [1], whereas CFs originating from endothelial cells by endothelial to mesenchymal transition (EndMT) are referred as endocardial fibroblasts [2]. Depending on their location and function, CFs can be broadly classified into interstitial septal and ventricular fibroblasts, adventitial septal and ventricular fibroblasts, atrial fibroblasts, annulus fibroblasts and cardiac valve fibroblasts [3]. CFs produce different components to maintain heart structure, such as periostin (Postn), vimentin, fibronectin and collagen types I, III, V and VI [4], and can be identified through certain markers such as cluster of differentiation 90 (CD90), discoidin domain receptor 2 (DDR2), fibroblast specific protein-1 (FSP-1), spinocerebellar ataxia type-1 (Sca1), fibronectin, vimentin and collagen types I and III [5]. Certain markers upregulate during cardiac fibrosis, such as fibroblast activating protein (FAP), fibroblast specific protein (FSP), fibronectin splice variant extra domain- A (ED-A), alpha smooth muscle actin (α-SMA), transcription factor 21 (Tcf21) and receptor tyrosine kinase platelet derived growth factor A (PDGFRα) [6]. Among these, α-SMA is the marker used for activated fibroblast identification. Tcf21 and PDGFRα are expressed by CFs during cardiac epicardial development [7]. CFs are distributed throughout the heart, where, predominantly present in the annulus fibrosus and adventia of coronary arteries and hence referred as valvular fibroblasts [8]. There are also more organized fibroblasts around the sinoatrial node which contribute to insulating the electric impulse [9]. Further, the fibroblasts are mainly present in coronary vessels [10]. The fibroblasts located between muscle fibres and referred as interstitial CFs and are less functional in the cardiac microenvironment [11]. CFs are the major source for ECM regulation, synthesis and degradation. They secret various collagens (I, III, IV, V and VI) to form ECM [12]. CFs, secrete a great number of proteins such as fibronectin, elastin, laminins, fibrillins, glycoprotein and proteoglycans, which are essential for ECM organization and cardiac signaling [13]. They also play roles as paracrine signals during the communication between CFs and other cardiac cells such as cardiomyocytes, endothelial cells and immune cells in the cardiac microenvironment [14]. ECM composition influences the response of fibroblasts to growth factors such as transforming growth factor beta (TGF-β) and induces its transition to myofibroblasts (MFBs) [15]. In myocardial infarcted heart, CFs at the initial stage of the inflammatory phase secrete matrix metallo proteinases (MMPs) which degrade ECM and promote cell migration to infarcted areas [16]. However, myocardial fibrosis induced by CFs plays a dual role in cardiac remodeling after injury. CFs are activated and deposit ECM at the injured part to heal the wound [13], but protracted inflammation can increase scarring and retard wound healing, causing cardiac dysfunction [17]. CFs are one of the major groups of cells present in cardiac tissue. In this review the development and function of CFs, and the crosstalk between CFs and cardiac stromal cells are discussed, especially the role of CFs

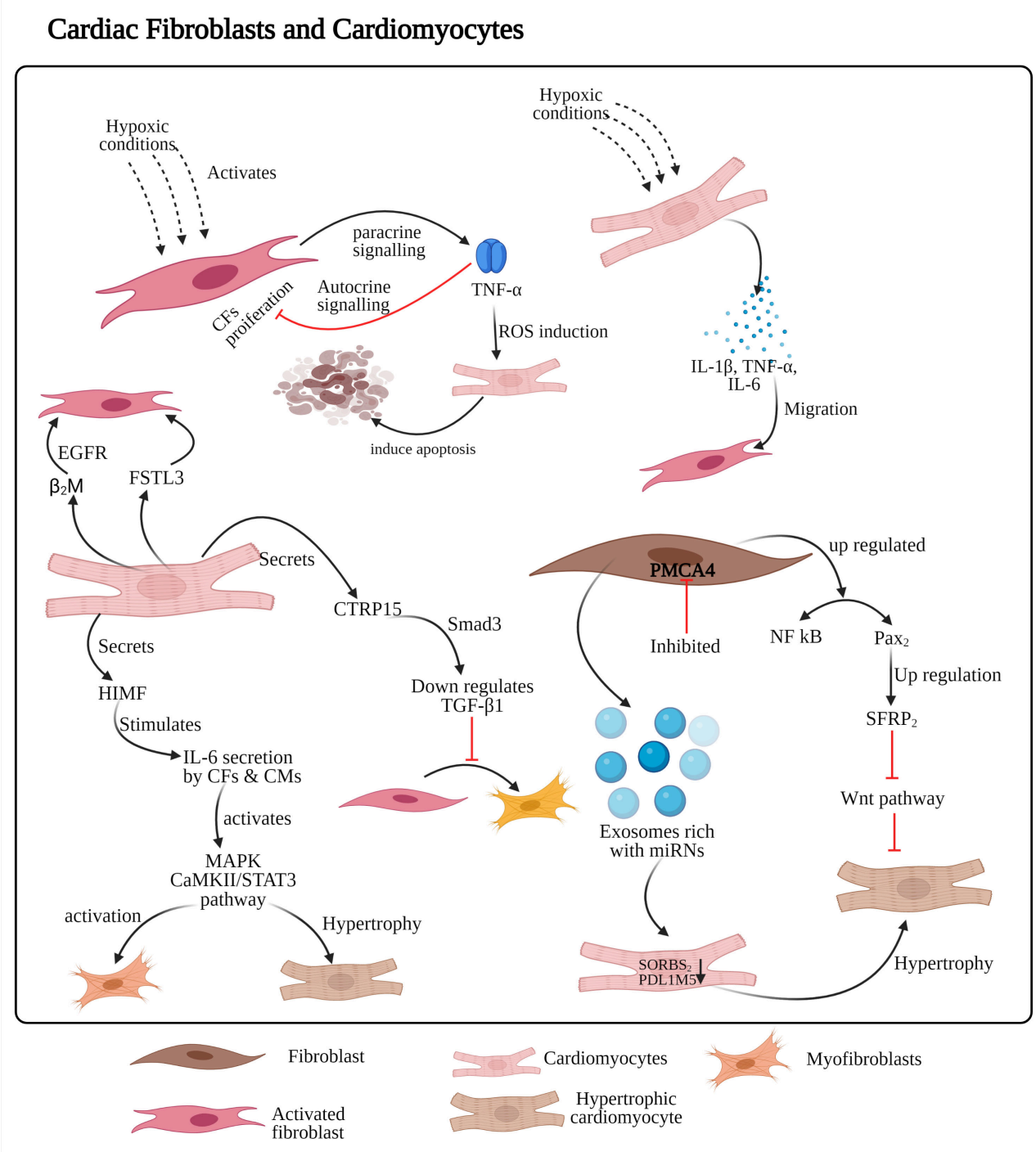

Fig. 1. Cardiac fibroblasts and cardiomyocytes interaction with and endothelial cells. CFs interact with cardiomyocytes either to promote or suppress the disease. CF autocrine and paracrine interaction by release of certain cytokines facilitates cell to cell communication. The secretions of other cardiomyocytes also influence fibroblast activation and attain myofibroblast phenotype.

functioning in electrophysiology and the induction of senescence. While several what we know about CFs has been derived from in vitro studies, there are also illuminating in future research. This review aims to raise a path to fill in the gaps of present studies, and to help better understand the role of CFs-in cardiac diseases.

2 Transition and differentiation of cardiac fibroblasts to cardiac myofibroblasts

CFs under the influence of several cardiac stressors such as physical stretch, cardiac stress, growth factors, and inflammatory mediators activate and convert to the MFB phenotype [18]. Prior to conversion of CFs to MFBs cells undergo an intermediate stage, the proto-myofibroblast phe-

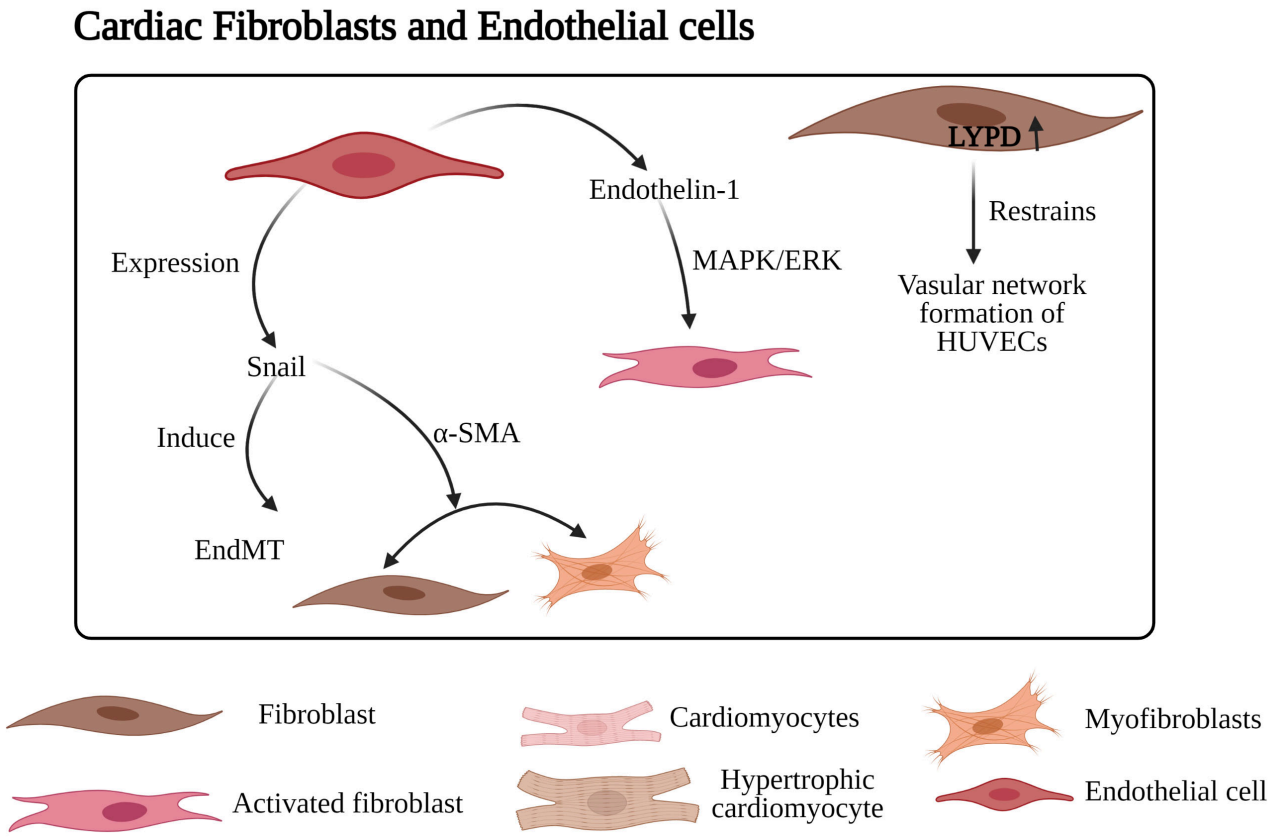

Fig. 2. Cardiac fibroblasts and endothelial cells interaction. Endothelin-1 and Snail expression by endothelial cells in the cardiac microenvironment activate fibroblasts and facilitate activated fibroblast conversion to myofibroblasts through α-SMA. Increased Ly6/PLAUR domain containing protein (LTPD) in fibroblasts restrains vascular network formation of human vascular endothelial cells (HUVEs).

notype. These, proto-myofibroblasts differ from MFBs by impaired expression of α-SMA [19]. These cells have migratory capacity by enhanced formation of stress fibers that facilitate contraction, while this phenomenon is not observed in non-activated fibroblasts [20]. CF conversion to MFBs is influenced by the TGF-β – Smad signalling pathway. TGF-β activates fibroblasts through phosphorylation of Smad2 or Smad3 and forming a complex with Smad4, then this complex translocates to the nucleus and subsequently binds and activates ECM genes such as *collagen-I* [18]. CF activation is also triggered by several growth factors such as connective tissue growth factor (CTGF/CCN2), angiotensin-II and platelet derived growth factor [21]. Certain intracellular signalling mediators such as, the a smad repressor- zinc finger E- box binding homeobox2 (Ski-Zeb2) – mesenchyme homeobox2 (Meox2) pathway, calcineurin/nuclear factor of activated T-cells (NFAT) and P38α induce transition of CFs to MFBs [22]. Apart from these, mechanical stretch of CFs transitions them to MFBs [23]. The differentiation between CFs and cardiac MFBs is challenging because of the lack of specific biomarkers. There are certain markers, such as vimentin, cluster of differentiation 31, 45 (CD31, CD45) and FSP-1, that are expressed by CFs and also by other cardiac cells such as, endothelial cells and macrophages [24, 25]. Also, there are certain markers that are expressed by CFs and cardiac MFBs such as transcription factor 21 (Tcf 21), osteopontin, frizzled-2 and DDR2 [12, 24, 26]. However, certain markers such as, platelet derived growth factor- alpha (PDGF-α), collagen1a1- green fluorescent protein (GFP) and cadherin-11 express on CFs but their expression on cardiac myofibroblasts needs to be clarified [24]. A few markers, such as transforming growth factor-β type-II receptor, angiotensin 1 receptor, tensin, paxillin, fibronectin, tenascin C and Postn show upregulated expression by MFBs during specific pathological or stress conditions [27]. However, currently α-SMA is used as the standard biomarker to differentiate cardiac MFBs and CFs because its expression is peculiar to stressed conditions and it is specifically not expressed by CFs [28]. Recent studies revealed a biomarker that is expressed on human CFs and cardiac MFBs, i.e., sushi containing domain 2 (SUSD2). Cultured CFs co-express SUSD2 with fibroblast marker PDGFRα [29].

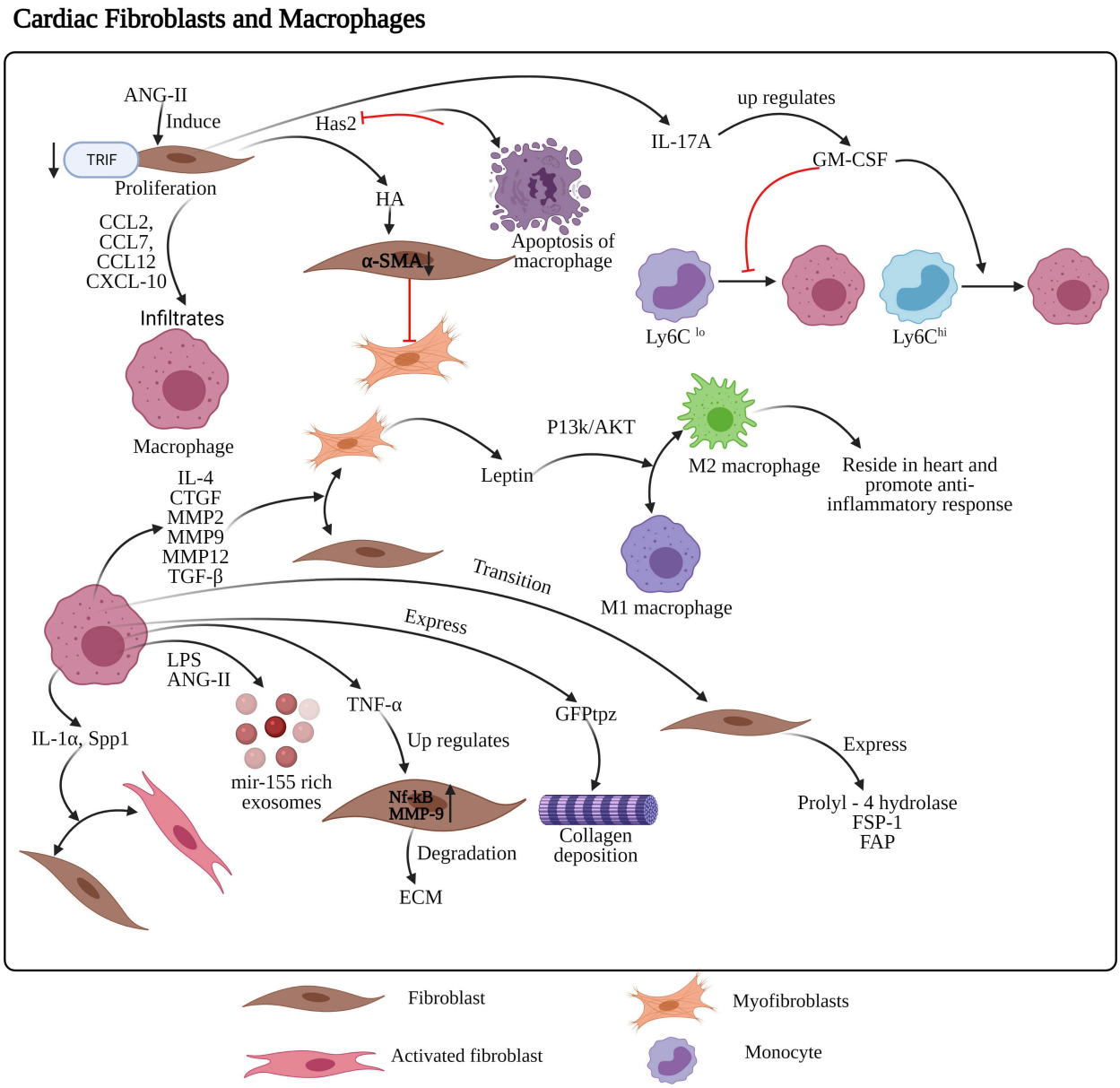

Fig. 3. Cardiac fibroblasts and cardiac macrophages interaction. CFs proliferates by stimulation with ANG-II and secretes certain cytokines that triggers infiltration of macrophages. Hyaluronan (HA) secretion downregulates expression of α-SMA and results in conversion of CFs to myofibroblasts. CFs secreted IL-17A upregulates GM-CSF that inhibits LY6C^{lo} monocytes conversion to macrophages and induces LY6C^{hi} monocyte conversion to macrophages. Cytokines secreted by macrophages convert CFs to myofibroblasts, leptin secreted by myofibroblasts converts M1 macrophages to M2 macrophages through P13K/AKT signaling. The secretion of IL-1α and Spp1 by macrophages causes activation of fibroblasts, TNF-α secretion upregulates NF-kB and MMP-9 expression in CFs and causes degradation of ECM. Green fluorescent protein transgene (GFPtpz) expression causes collagen deposit.

3. Cardiac fibroblasts in development of pre-natal and post-natal heart

Cardiac development, also referred to as cardiogenesis, is an important physiological process during mammalian embryonic development. The heart is the first functional organ developed during embryonic development [30]. At the 4th week of gestation, epicardial epithelial cells undergo EMT and form CFs and the vascular smooth muscle cells of matured hearts [31]. Cardiomyocytes, CFs, endothelial cells and valvar interstitial cells are the major cardiac cells during embryonic heart development [32]. Cells expressing genes such as *collagen type 1 alpha* (*COL1A1*), *COL1A2*, *COL3A1*, *Postn and decorin* (*DCN*) are represented as CFs in the embryonic heart. Their main function is formation of ECM [13]. CFs express four different gene groups during the developmental stage. The first associates with striated muscle cell development, with expression upregulated in mid and late stages of development [33]. At 17 weeks gestational stage development, fibroblasts express SRY-Box transcription factor-9 (SOX9) a

sarcomere protein, i.e., cardiac troponin T [33]. At this stage there is a severe change in the cell cycle. The expression of cell cycle genes is significantly reduced during development suggesting the reduction of CF proliferation during heart development. The mitotic genes *aurora kinase B* (*AURKB*), *cyclin dependent kinase 1* (*CDK1*) and *ubiquitin conjugating enzyme* E_2C (UBE_2C) are downregulated, together with the upregulation of ECM deposition related genes such as *DCN* and *COL3A1* etc., showing upregulated expression in the early- to mid-stage of heart development [33]. Thus ECM deposition is essential to maintain the structure and promote cell to cell interaction in the cardiac microenvironment. ECM expression is upregulated in week 5 to week 6 of the gestational period. Furthermore, fibroblasts could upregulate the expression of the bone morphogenic protein (BMP) signaling pathway during development [33] through increasing the expression of BMP signaling pathway receptors such as bone morphogenic protein receptor type 2 (BMPR2), activins A receptor (ACVR), drosophila mothers against decapentaplegic protein $(SMAD)_5$ and $SMAD_9$ which are essential for cell to cell communication during cardiac development [33].

During postnatal development the interactions between the cells in the cardiac microenvironment through several signaling pathways contributes to heart development and disease [34]. CFs facilitate cardiomyocyte maturation by upregulated expression of certain genes such as *DCN*, *laminin subunit gamma 1* (*LAMC1*), *placental growth factor* (*PGF*) and *laminin subunit alpha-2* (*LAMA2*) to secrete maturation promoting proteins which can bind to the receptor on cardiomyocytes and promote maturation [35]. Furthermore, the BMP signaling pathway between CFs and cardiomyocytes initiates maturation of cardiomyocytes prominently, and there are other common pathways activated by cardiac fibroblasts such as fork head box O (FOXO), mechanistic target of rapamycin (mTOR), and vascular endothelial growth factor (VEGF) that contribute to switching of the heart from neonatal to postnatal matured heart [35]. There are critical changes in heart following birth. Heterogeneous cardiac fibroblasts populations exist from the third day after birth. These fibroblasts express different endogenous genes such as *Postn* and *Tcf21*. α-SMA is also expressed by MFBs, which is essential for ECM remodeling after birth. Postn's expression is persistent until postnatal day 30 (P30), but is downregulated when Tcf21 is expressed. Based on these gene expressions, CFs are of these groups: Postn expressing at postnatal day 7 ($Postn^+$P7), Tcf21 expressing at postnatal day 7 ($Tcf21^+$P7) and Tcf21 expressing at postnatal day 30 ($Tcf21^+$P30). $Postn^+$P7 and $Tcf21^+$P7 are no longer detected at P30, but $Tcf21^+$P30 CFs downregulates ECM expression and remains quiescent at P30. $Postn^+$ CFs also remain quiescent but are activated after injury to perform cardiac repair. There is a need to know $Postn^+$ CFs' interaction with other CFs in cardiac microenvironment in the repair mechanism [36].

4. The cross talk between fibroblasts and cardiac stromal cells

The cardiac microenvironment is composed of several cell types: 60–70% of non-myocytes such as endothelial cells, vascular smooth muscle cells and fibroblasts; 30–40% of cardiomyocytes responsible for the contractility of heart muscles [37]. These cells interact with each other due to alterations in development, homeostasis and pathological triggers.

4.1 Cardiac fibroblasts and cardiomyocytes

CFs and cardiomyocytes interconnect through cell-to-cell communication directly or indirectly by expressing cytokines and other mediators via the autocrine or paracrine pathway during pathological stress [38]. In *in vitro* conditions, expression of tumor necrosis factor alpha (TNF-α) in CFs was upregulated under hypoxic conditions, instead of interleukin 1 beta (IL-1β), IL-10 and interferon gamma (INF-γ), to reduce the threshold for mitochondrial permeability transition (MPT) induction by reactive oxygen species (ROS) in cardiomyocytes, causing the apoptosis of cardiomyocytes and the decrease of CF proliferation, in turn resulting in aggravation of ischemia reperfusion injury (Fig. 1) [39]. Meanwhile, hypoxic cardiomyocytes secrete some metabolites like IL-1β, TNF-α and IL-6, among them IL-1β and TNF-α depending on *in vitro* exposure time intervals, contributing to fibroblast migration prior to myocardial damage. Prolonged hypoxic conditions upregulate transforming growth factor beta (TGF-β), a pleiotropic cytokine that contributes to cardiac repair and remodeling by significant inhibition of fibroblast migration [40, 41]. C1q/tumor necrosis factor related protein (CTRP) expression is compromised in cardiomyocytes of the left ventricle of mice that underwent transverse aortic constriction (TAC), but in pressure overloaded mice CTRP15's expression is upregulated by adeno associated virus (AAV9), which contributes to downregulation of profibrotic molecule TGF-β1 through the Smad3 pathway. Moreover, CTRP15 augments the phosphorylation of insulin receptor (IR) and causes the activation of protein kinase B (AKT), which results in an antifibrotic effect (Fig. 1). There is a need to elucidate the mechanism in upregulation of IR by CTRP15 [42]. However, in clinical studies β_2M (a non-glycosylated protein) over expression by cardiomyocytes promotes the activation of CFs through epidermal growth factor receptor (EGFR) signaling under pressure overload, which causes cardiac dysfunction in hypertension and heart failure patients [43]. Metabolites such as hypoxia induced mitogenic factor (HIMF) overexpression in cardiomyocytes influences the activation of CFs. Moreover, in mice HIMF also stimulates IL-6's secretion of CFs, but blocking of IL-6 downregulates HIMF's expression. IL-6 in cardiomyocytes and fibroblasts activated mitogen activated protein kinase (MAPK) and calcium/calmodulin dependent kinase II (CaMKII)-signal transducer and the activator of transcription 3 (STAT3) pathway, which stimulates activation of fibroblasts and hypertrophy of cardiomy-

ocytes resulting in promotion of fibrosis and cardiac hypertrophy [44]. Follistatin-like 3 (FSTL3) was overexpressed by cardiomyocytes under a stress condition, which induced CF activation and proliferation and led to progression of disease [44]. α-SMA negative fibroblasts in normal heart cause reduction of adult cardiomyocyte viability through paracrine signaling, but cardiomyocytes, along with TGF-β, secrete other metabolites that affect CF proliferation [45]. In mice, the suppression of plasma membrane calcium ATPase 4 (PMCA4)'s expression in fibroblasts causes increased expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and paired box gene 2 (Pax2), where Pax2 is the major transcription factor for secreted frizzled related protein 2 (SFRP2) expression, and its upregulation would inhibit the Wnt pathway and then suppress hypertrophic responses in cardiomyocytes. Here, there is a need to know PMCA4 action on endothelial and smooth muscle cells, which could further contribute to therapeutics [46]. CFs secrete star microRNA (miRNAs) enriched exosomes due to stress signals, and these exosomes act upon cardiomyocytes by paracrine signaling to downregulate expression of sorbin and SH3 domain containing 2 (SORBS2) and PDZ and LIM domain 5 (PDLIM5) (Fig. 1). Under normal conditions, SORBS2 is essential for the assembly of myofibrils and several other important processes in cardiomyocytes, and PDLIM5 is required for maintenance of cardiac muscle structure and function. So the downregulation of SORBS2 and PDLIM5 by miR-21 would contribute to cardiac failure caused by myocardial hypertrophy [47]. *In vitro* and *in vivo* studies reveal that cardiomyocytes secreted exosomes containing miR-208a were up taken by CFs. These exosomes induce proliferation of fibroblasts and also trigger their transition to MFBs. These effects are mediated by miR-208a through upregulation of dual specificity tyrosine phosphorylation regulated kinase-2v (DYrk2) expression that induces phosphorylation of NFAT, causing cessation of its entry into the nucleus resulting in induction of fibrosis [48].

4.2 Cardiac fibroblasts and endothelial cells

Endothelial cells (ECs) are an elementary part of the vasculature and the heart, where cardiac ECs could modulate performance of cardiac muscles [49]. Endocardial ECs release cytokines like TGF-β, Endothelin 1 (ET-1) and angiotensin II (Ang-II). Among these, ET-1 stimulates CF proliferation via the MAPK/(mitogen activated protein kinases/extracellular signal-regulated kinases) ERK pathway, which is involved in the progression of fibrosis by collagen synthesis (Fig. 2) [50]. Zinc finger protein SNAl1 (Snail) expressed by endothelial cells could induce EndMT and stimulate differentiation of CFs to MFBs in a mouse ischemia reperfusion injury model (I/R injury). Meanwhile, the expression of connective tissue growth factor (CTGF) as a profibrotic factor is also upregulated in correspondence with Snail, following the secretion of α-SMA and smooth muscle 22 alpha (SM22α) as mesenchymal components to induce the migration of fibroblasts (Fig. 2) [51]. However, Ly6/PLAUR domain containing protein (LYPD) upregulated expression in CFs restrains vascular network formation of human vascular endothelial cells (HUVECs) [51], which exhibit cardiac dysfunction caused by the interaction between CFs and endothelial cells. In recent studies TGF-β activated CFs secrete altered exosomes, i.e., miR-200a-3q which modifies angiogenic potential, proliferation and migration of endothelial cells. These exosomes regulate phosphatidylinositol glycan anchor biosynthesis class F (PIGF)-dependent vascular endothelial growth factor A(VEGF-A) signalling which induces dysfunction of endothelial cells. Inhibiting miR-200a-3q in MFBs restores endothelial function [52]. There is a need for further exclusive study of several autocrine and paracrine factors and mechanisms that are hidden in the interaction between CFs and endothelial cells.

4.3 Cardiac fibroblasts and cardiac macrophages

Macrophages are the key cells that induce inflammation in cardiac injury and cardiac repair [53]. CFs and macrophages interact with each other through several cytokines' paracrine activity [54]. Studies on patients with rheumatic heart disease with mitral/aortic valve replacement discover that Ang-II induced fibroblast proliferation caused by upregulated expression of Toll/IL-1 receptor domain containing adaptor (TRIF) during atrial fibrillation. Upregulated TRIF could increase macrophage infiltration into atrial fibroblasts by chemotaxis induction via c-c motif Chemokine ligand 2 (CCL2), CCL7, CCL12 and c-x-c motif Chemokine ligand 10 (CXCL10). These macrophages associate with atrial fibroblast proliferation and result in fibrosis [55]. In myocardial infarction (MI) induced mice, there is an increased number of $CD206^{+}F4/80^{+}CD11b^{+}$ M2-like macrophages stimulating CFs transformation into MFBs through increasing the expression of IL-4 post MI. Simultaneously, IL-1α and osteopontin (Sppl) are also nearly 3-fold upregulated to stimulate the activation of fibroblasts and result in repair of post-MI hearts [56]. In mice, induced acute myocardial infarction (AMI) studies revealed that mir-155 upregulated expression in macrophages is influenced by lipopoly saccharides (LPS) and Ang-II. Activated macrophages release exosomes enriched with mir-155, which inhibits cardiac fibroblast proliferation by suppressed expression of son of seven less homolog 1 (Sos1) protein causing impaired cardiac repair after MI (Fig. 3). Sos1 is a prominent protein that interacts with growth factor receptor bound protein 2 (Grb2) and stimulates cell proliferation by activating ERK [57]. Clinical studies on cardiac rupture (CR) in AMI reveal that S100A8/A9 is overexpressed by macrophages in peripheral blood and myocardial tissue. S100A8/A9 interacts with advanced glycation end products (RA-GE) and Toll-like receptor 4 (TLR-4) stimulates migration and activation of macrophages. Moreover, TNF-α overexpressed by macrophages causes upregulated expression of NF-kB and matrix metallopeptidase 9 (MMP-9) in human CFs leading to degradation of ECM followed by CR [58]. Particularly, our studies on experimental autoimmune myocarditis (EAM)

and viral myocarditis-induced mice reveal that cardiac resident macrophages play a prominent role in homeostasis, maintenance of cardiac function and tissue repair. Ang-II induces transdifferentiation of CFs to MFBs, and these MFBs could secrete leptin to promote M1/M2 macrophages conversion through the phosphoinositide-3-kinase (PI3K) or the AKT pathway (Fig. 1). M1 macrophages perform apoptosis through the TNF/tumor necrosis factor-1 (TNFR1) axis resulting in suppression of inflammation. Simultaneously, converted M2 macrophages reside in the heart and promote anti-inflammatory responses [59]. In myocarditis-induced mice, high expression of IL-17A in CFs stimulates increased expression of granulocyte macrophage colony stimulating factor (GM-CSF) which suppresses lymphocyte antigen 6 low ($Ly6C^{lo}$) monocyte differentiation to macrophages, but activates lymphocyte antigen 6 high ($Ly6C^{hi}$) monocytes derived macrophage (MDMs), and causes activation of inflammation, promotion of tissue remodeling and reduction of phagocytosis, and thereby myocarditis related heart failure (Fig. 3) [60]. In a transverse aortic constriction (TACn) mouse model, $LY6C^{hi}$ monocytes accumulate in cardiac hypoxic areas by hypoxia inducible factor 1-alpha (HIF-α) signaling, which overexpress oncostatin-m (OSM) to target TGF-β1 mediated CF activation through extracellular signal regulated kinase1/2 dependent phosphorylation of the SMAD liker region. Thus OSM could suppress excessive fibrosis in hypoxic cardiac tissue [61]. Studies in a cardiac injury mouse model reveal that macrophages express collagen and collagen associated genes that caused collagen fiber deposition in forming scar, which suggests that macrophages participate directly in cardiac fibrosis [62]. In the mouse model, different macrophage subsets show different effects on CFs in cardiac fibrosis. M_2a, M_2c and M_0 phenotype show antifibrotic activity, but M_2b and M_1 phenotype show profibrotic activity. M_2a macrophages trigger cardiac fibroblasts to express CTGF, and then cause proliferation, migration, and differentiation to MFBs. M_2c macrophages accelerate M_2a function through upregulated expression of α-SMA. However, M_2b macrophages exert roles opposite to those of M_2a and M_2c, even though M_2b macrophages show beneficial action in the early stage after myocardial I/R injury by regulating MAPK signaling. Late stage M_2b macrophages are dominated by M_1 macrophage and cause severity of the disease. There is a need to elucidate the relation between M_2b and MAPK signaling and CFs, so it is necessary to clarify M_2b interrelations with other signaling pathways [63]. CFs secrete hyaluranan (HA) by Has_2 in I/R injury, where the down-regulation of Has_2 caused reduction of α-SMA positive fibroblasts, resulting in decrease of MFB differentiation and proliferation (Fig. 3), and the blockade of CD44 as HA's receptor would inhibit TGF-β1-specific responses like SMAD2 phosphorylation, causing hampered secretion of α-SMA secretion. Moreover, the decrease of hyaluronan synthase-2 (Has_2) could induce apoptosis of macrophages, which indicates that HA plays a prominent role in post-infarct healing [64]. Macrophages could upregulate expression of TGF-β and metalloproteinase such as MMP2, MMP9 and MMP12 in Chagas disease (CD) caused by *Tryoanosomacruzi,* due to differentiation of CFs to MFBs. Upregulation of poly [adenosine diphosphate ribose (ADP)-ribose] polymerase 1/activator protein-1 (PARP1/AP-1) in infected macrophages results in transcriptional activation of MMP/TGF-β responses in macrophages by c-Fos and Jun B mediated AP-1, followed by development of cardiac fibrosis [65]. Studies in mice have confirmed the phenotypic transition of infiltrating macrophages to fibroblastic-like cells post MI, according to the upregulation of fibroblast markers such as type-I collagen, prolyl-4-hydroxylase, fibroblast specific protein-1 and fibroblast activation protein in macrophages in the heart after MI. Macrophage transition to fibroblasts improves cardiac regeneration after MI. In this regard, continued research into the transition of tissue resident macrophage merits further exploration [66].

5. Cardiac fibroblasts in cardiac electrophysiology

Electrophysiology is a test performed to understand proper electrical functioning of the heart. In the heart, atrial and ventricular muscles follow a synchronized pattern of rhythmic contraction and relaxation, represented as depolarization and repolarization of heart due to electrical activity [67]. These electrical signals are generated from the sinoatrial node (SA), also referred as the pacemaker of the heart. These electrical activities of the heart are recorded by a medical device referred to as an electrocardiograph (ECG) [68]. Normally, the major cardiac cell groups such as cardiomyocytes actively contribute to the electrophysiology of the heart, while CFs as non- electrical cells or non- beating cells could decrease the synchronization of cardiomyocytes [69]. CFs can connect cardiomyocytes through intercellular communication and decrease the velocity of electrical signaling. Excessive CFs cause less synchronization, influencing the rhythm of the heart beat [70]. Upon cardiac injury, healing progresses by cardiomyocytes as opposed to CFs. CFs due to injury convert to α-SMA expressing MFBs and result in deterioration of heart function. Here, connexin 43 is qualitatively observed proving the cell to cell communication [71]. CFs co-cultured with sinoatrial nodal cells (SANCs) can beat in a synchronized manner and also express proteins like cardiac troponin T (cTnT) and desmin. In these studies, prolonged co-culture of SANCs and CFs result in the homeoprotein expression of NK2 homeo box 5 (Nkx2.5), which is a protein exclusively expressed by cardiomyocytes. These studies hypothesize that SANCs could help CFs transdifferentiate to cardiomyocytes and beat, with eventual loss of pulsatility, which may be due to Nkx2.5 loss of binding to its receptor. To prove this hypothesis there is a need of intensive study on early stage differentiation to cardiomyocytes [72]. Heart failure (HF) is the cause of highest mortality. Several HF-biomarkers such as, amino terminal pro-B type natriuretic

peptide (NT-pro BNP), galectin-3 (gal-3) and soluble(s) ST2, are used for assessment of acute HF clinically by collecting peripheral venous (PV) samples [73]. In chronic heart failure (CHF) by left ventricular remodeling was because of several cytokines such as, IL-1-β, IL-6 were elevated in coronary sinus (CS) serum than PV of CHF patients, this indicated that these cytokines were involved in heart failure. Where, NT-pro BNP concentration also increased in the samples obtained from left ventricle in CHF patients. This suggests that ventricular secretion of NT-proBNP cause CHF. Whereas, inflammatory stimulation of pericardial mesothelial cells increases secretion of carbohydrate antigen 125 (CA125) both in CS and PV. Hence, not only PV but CS level of CA125 and NT pro BNP would be used as biomarkers in CHF patients [74]. To overcome HF there is device therapy, i.e., cardiac resynchronization therapy (CRT). However, 1/3rd of patients are non- responsive to CRT, hence there is a need to evaluate biomarkers responsible for HF even after CRT. Usually, samples were collected from PV to analyse biomarkers but sampling from the (CS) is more prominent and useful than PV sampling. In the CS, biomarkers such as NT-pro BNP, gal-3 and sST2 are significantly elevated, so it would possess a better prognostic role [75].

6. Cardiac fibroblasts and cardiomyocytes senescence

Cellular senescence is the occurrence of cell cycle arrest at G1 phase and is irreversible. It plays a prominent role in physiological and pathological processes [76]. Adult CFs would decrease the electrophysiological and mechanical function of co-cultured neonatal rat ventricular monocytes (NRVMs) through downregulating the expression of ion channels, electrical coupling, calcium handling and contraction related genes in the cardiac microenvironment [77]. Phenolic compounds (PCs) protect the heart from age-related detrimental effects in age-related cardiac remodeling. PCs could ameliorate several hypertrophic pathways such as calcineurin/nuclear factor of activated cells (NFATc3), CAMKII, extracellular regulated kinase ½ (ERK ½) and glycogen synthase kinase 3β (GSK3β). Along with these effects, PCs reduce the expression of plasma inflammatory and fibrotic markers. The P38 pathway is regulated to ameliorate ECM remodeling. PCs exhibited reduced fibrosis by downregulation of the pro-fibrotic TGF-β1/Smad pathway [78]. Recent research indicated that there exists senescence of CFs in a post-natal day 1 (P1) neonatal mouse heart apical resection (AR) model, but the senescent cells disappear at P21 when the hearts were fully restored. Matricellular protein cellular communication network factor-1 (CCN1) has upregulated expression in cardiomyocytes at P4 after AR. Knock down of CCN1 would decrease cardiomyocytes and increase CFs. CCN1 is essential for senescence induction of CFs and also helps in secretion of senescence associated secretory phenotype factors such as IL-1a and IL-6 [79]. In infarcted or hypoxia treated heart, fibroblasts show upregulated expression of P53, which is a senescent cell target gene [80]. Ischemia and hypoxia-induced downregulation of sirtuin 1 (Sirt1) and neonatal rat cardiomyocytes (NRCMs) in mouse heart causing worsening of heart function. Resveratrol (RSV) could reverse Sirt1 expression and cause P53 deacetylation resulting in reduction of senescent cardiomyocytes [81]. Plasminogen activator inhibitor-1 (PAI-1) is a prominent agent in the induction of cellular senescence during aging and pathological conditions. TM544 is the small molecule inhibitor of PAI-1 which inhibits cellular senescence caused by doxorubicin (an anti-cancer drug) in cardiomyocytes, fibroblasts, and endothelial cells, followed by the amelioration of anti-cancer-treatment-induced cardiac toxicity [82]. Senescent cardiac fibroblasts express serine protease inhibitor E1 (SERPINE1), which regulates functional activity of cardiac endothelial cells through deregulation of angiogenesis resulting in progression of cardiac dysfunction [83].

7. Conclusions

CFs are a major component in the cardiac microenvironment. Their interaction with cardiomyocytes and macrophages has been well studied, but the mechanisms need to be further elucidated. Improved understanding of cardiac development helped define some of these cues. Postn$^+$ CFs that reside in cardiac tissue as quiescent cells can be activated after injury, but the relation and interaction between Postn$^+$ CFs and other CFs in cardiac tissue need to be elucidated. CFs acquire pulsatility when co-cultured with SANCs, but this transition did not show persistence per electrophysiology. The mechanisms and factors involved in the transition, which would contribute to persistent beating of early cardiomyocytes formed from CFs, need to be elucidated. Moreover, the relationship between cardiomyocyte senescence and CFs merit particular scientific attention. CCN1's function is well studied in neonatal heart. Elucidation of its function in adult heart could contribute to future therapeutics.

Author contributions

RSL and LX drafted the manuscript under the supervision of FL, who edited and approved the final version. RSL and YPZ drafted all figures. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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