REVIEW ARTICLE

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Signaling pathways in cardiac fibrosis; A review and future therapeutic options

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Abstract

Myocardial fibrosis refers to a variety of quantitative and qualitative changes in the interstitial myocardial collagen network that occur in response to cardiac ischemic insults, systemic diseases, drugs, or any other harmful stimulus affecting the circulatory system or the heart itself. Myocardial fibrosis alters the architecture of the myocardium, facilitating the development of cardiac dysfunction, also inducing arrhythmias, influencing the clinical course and outcome of heart failure patients. Focusing on myocardial fibrosis may potentially improve patient care through the targeted diagnosis and treatment of emerging fibrotic pathways. The current review highlights the most important signaling pathways involved in the pathogenesis of cardiac fibrosis. Targeting these pathways is the key objective in introducing new therapeutic modalities to protect myocardium from remodeling and fibrosis. Present work also highlights new options currently being tested and used in mitigating fibrosis in heart. One of these options is the use of gliflozins, relatively new oral hypoglycemics, which show promising cardioprotective effects. Gliflozins are Sodium-glucose cotransporter 2 inhibitors (SGLT2i), a new drug class approved for treatment of diabetes, which have been shown to possess a favorable metabolic profile and to significantly reduce atherosclerotic events, hospitalization for heart failure, cardiovascular and total mortality, and progression of chronic kidney disease. Although initially considered to be only glucose-lowering agents, the effects of SGLT2i have expanded far beyond that, and their use is now being studied in the treatment of heart failure and chronic kidney disease, even in patients without diabetes.

Keywords: Cardiac fibrosis; Pirfenidone; Empagliflozin; TGF-β; SMAD.

1. Introduction

In adult mammals, the heart possesses only a restricted ability to regenerate, underscoring the significance of reparative mechanisms following injury. These mechanisms entail the infiltration of inflammatory cells, the elimination of necrotic heart muscle cells, and the development of granulation tissues rich in capillaries. Subsequently, fibrotic scars take the place of granulation tissues, serving to maintain the structural and functional integrity of the myocardium. Fibrosis is characterized by the overaccumulation of extracellular matrix (ECM) components, predominantly collagen fibers, in the interstitial spaces. It represents a crucial pathological response to chronic inflammation. **(Maruyama and Imanaka-Yoshida, 2022)**.

Initially, the deposition of extracellular matrix (ECM) proteins serves a protective role and is essential for wound healing and tissue regeneration. The ECM also plays a crucial role in maintaining normal physiological conditions. For example, interactions between cardiomyocytes and the ECM influence cellular behavior through cell surface receptors, which function as signal transmitters governing processes like cell proliferation, migration, survival, and differentiation **(Sainio and Järveläinen, 2020)**. Nevertheless, when pathological cardiac remodeling occurs due to excessive and persistent tissue damage, accompanied by ongoing ECM deposition, it leads to an altered organ structure and has a profound impact on cardiac function **(Li et al., 2018; Frangogiannis, 2021)**.

Cardiac fibrosis is seemingly related to common risk factors e.g. genetic, viral, and environmental abnormalities **(Hinderer and Schenke-Layland, 2019)**, and it was also linked to major health problems e.g. obesity, hypertension, diabetes, and metabolic dysfunction **(Cavalera et al., 2014)**. Heart diseases, including conditions like myocardial infarction (MI), cardiac hypertrophy resulting from pressure or volume overload, diabetic cardiomyopathy, and dilated cardiomyopathy (DCM), collectively contribute to the progression of cardiac fibrosis. While fibrosis holds significant pathophysiological relevance in the development of cardiovascular diseases, there remains a need for further research to fully understand the mechanisms and processes involved in cardiac fibrosis development at the molecular level **(Krejci et al., 2016; Frangogiannis, 2021; Imanaka-Yoshida, 2021)**.

The prevailing approach for diagnosing cardiac fibrosis involves identifying and measuring the amount of collagen in the interstitial tissue, typically through an endomyocardial biopsy **(Jellis et al., 2010)**. Meanwhile, the most widely used technique for assessing left ventricular (LV) volume and mass is cardiac magnetic resonance (CMR) imaging. Nevertheless, CMR is a costly method that demands expertise for both image acquisition and analysis **(Graham-Brown et al., 2017)**. To identify perfusion irregularities or discrepancies in metabolism and perfusion, nuclear imaging techniques such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) have been employed. These methods serve specific purposes: SPECT for detecting perfusion defects and PET for assessing metabolism and perfusion mismatches **(Hinderer and Schenke-Layland, 2019)**.

In addition to imaging approaches, non-invasive methods for detecting fibrosis also exist, involving the use of biomarkers. For instance, the ratio of matrix metalloproteinase type 1 to tissue inhibitor of metalloproteinase type 1 (MMP-1/TIMP-1) or the measurement of the carboxy-terminal pro-peptide of pro-collagen type I (PCIP) in the bloodstream are commonly utilized **(Jellis et al., 2010)**. Currently, all of these methods and techniques are in regular use. However, none of them satisfies all the criteria for identifying myocardial fibrosis comprehensively. Therefore, it is typically necessary to combine imaging, biomarker assessment, and routine histological and histochemical staining to fully characterize cardiac fibrosis.

Numerous signaling pathways have been implicated in the activation of cardiac fibroblasts (CFs) and the progression of pathological remodeling. Exploring the modulation of these signaling pathways as potential novel therapeutic targets holds significant promise. These signaling pathways will be shortly summarized in this review, alongside with therapeutic modalities present in combating against cardiac fibrosis up to date.

2. Signaling Pathways of Cardiac Fibrosis

2.1. TGF-β Signaling

Transforming growth factor-β (TGF-β) is the most

pleiotropic peptide exerts a wide range of effects, but its impact on cellular behavior varies depending on the specific cell type, the surrounding environment, and the cellular conditions. Importantly, TGF-β's actions are highly context-dependent and can differ significantly based on the circumstances in which it operates **(Zhang et al., 2017)**. TGF-β facilitates various biological processes, such as embryonic development, tumor growth, cell proliferation, and apoptosis (**Zinski et al., 2018; Batlle and Massagué, 2019; Tzavlaki and Moustakas, 2020)**.

The TGF-β is also a central player in hypertrophic and fibrotic transformation of the heart, mediating cardiomyocyte growth, CF activation, inflammation, and ECM deposition **(Liu et al., 2017; Liu et al., 2019a)**. TGF-β includes three isoforms (TGF-β1, TGF-β2, and TGF-β3) in mammals, encoded by three different genes **(Tzavlaki and Moustakas, 2020)**. Among the three isoforms, TGF-β1 is predominant. It is crucial in pathological fibrosis and is produced by various cells, including immune cells, endothelial cells, cardiomyocytes, and activated fibroblasts **(Hanna and Frangogiannis, 2019; Nicin et al., 2022)**. TGF-β1 is initially secreted as an inactive complex with dormant TGF-β-binding proteins and TGF-β pro-peptides. This complex is sliced and activated during an integrin-mediated process **(Robertson and Rifkin, 2016; Brown and Marshall, 2019)**.

The TGF-β can induce signal transduction via primary (SMAD-dependent) and non-primary (SMAD-independent) pathways. In the canonical pathway, TGF-β1 binds to and causes heterodimerization of TGF-β receptor type 1 (TβRI, also known as activin-like kinase (ALK) 5) and type II (TβRII), leading to SMAD2 and SMAD3 phosphorylation. Consequently, a complex with SMAD4 forms and translocates into the nucleus, acting as a transcriptional factor to regulate the expression of target genes **(Chung et al., 2021)**. Intriguingly, recent reports have suggested the distinct roles of SMAD2 and SMAD3 in mediating TGF-β signaling **(Aragón et al., 2019; Huang et al., 2019)**. SMAD6 and SMAD7 are inhibitory SMADs. They can interact with TβRI and competitively inhibit SMAD2 and SMAD3 **(Chung et al., 2021)**. In addition to the SMAD-dependent canonical pathways, TGF-β1 can induce SMAD-independent non-canonical signaling that involves several mitogen-activated protein kinases, including extracellular signal-regulated kinases (ERKs), c-Jun extensively studied agent as a fibrotic factor. This 1 (TAK1), Rho family of small GTPases, and p38 MAPK pathways **(Zhang, 2017; Frangogiannis, 2020)**.

In fibrosis regulation, TGF-β can transform fibroblasts into activated CFs and promote ECM synthesis and deposition, which involves SMAD3 signaling **(Khalil et al., 2017)**. TGF-β also inhibits ECM degradation by regulating plasminogen activator inhibitor (PAI)-1 and TIMP expression levels **(Schiller et al., 2004)**. Additionally, non-canonical TGF-β signaling can induce fibrosis. In human activated CFs, RNA-binding proteins, such as pumilio RNA binding family member 2 (PUM2) and KH domain-containing RNA binding (QKI), work as hub proteins of the canonical TGF-β1–SMAD and TGF-β1–MAPK pathway, and the non-canonical IL-11-mediated pathway, which regulates fibrogenic gene expression **(Chothani et al., 2019; Finnson et al., 2020)**. Extensive and accumulating evidence highlights the significance of non-coding RNAs, including microRNAs, in the development of cardiac fibrosis **(Micheletti et al., 2017; Yousefi et al., 2020)**. TGF-β signaling pathways in cardiac fibrosis are illustrated in **Figure 1**.

2.2. Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system, in which Ang II is considered the most predominant isoform, promotes many pathophysiological functions, including cardiac fibrosis **(Jia et al., 2018)**. Ang II can be produced either systemically or locally and exerts its effects through two specific receptors: angiotensin type 1 (AT1) and type 2 (AT2). AT1 receptor activation is associated with various biological processes, including the proliferation and migration of cardiac CFs, CF activation, the synthesis of ECM proteins, and apoptosis. In contrast, AT2 receptors play a cardioprotective role and act as negative regulators of Ang II-mediated fibrogenic responses. They achieve this by inhibiting AT1 receptor actions, which leads to the suppression of CF proliferation and matrix synthesis **(Paz Ocaranza et al., 2020)**.

The effects of Ang II through AT1 on CF activation are mediated through the activation of p38 MAPK, protein kinase C (PKC), and ERK cascades **(Forrester et al., 2018)**. Ang II also interacts with TGF-β signaling in cardiomyocytes and CFs to induce cardiac hypertrophy and fibrosis.

N-terminal kinases (JNKs), TGF-β-activated kinase Various mediators regulate CF responses to Ang II

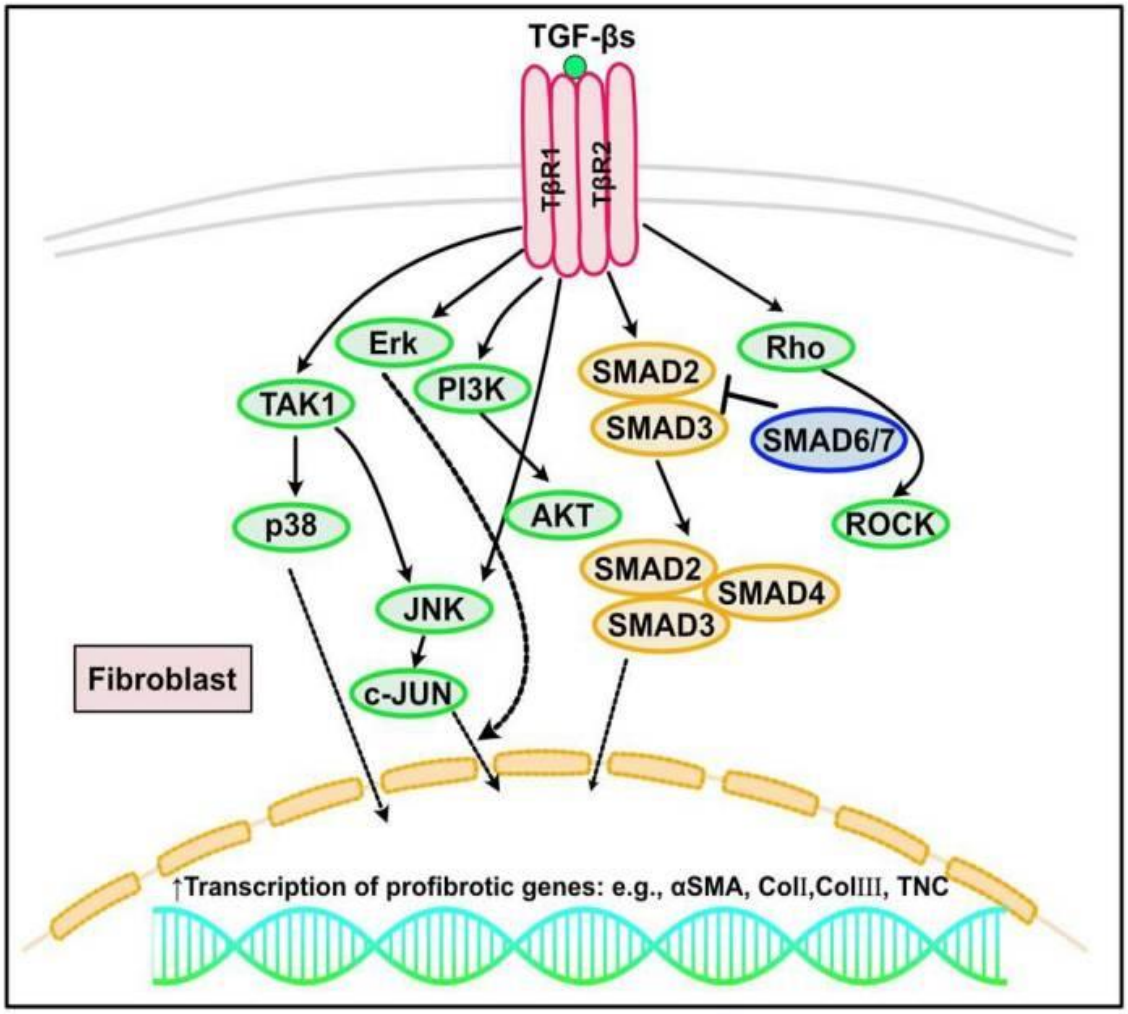

Figure 1: TGF-β signaling in cardiac fibrosis **(Maruyama and Imanaka-Yoshida, 2022).** TGF-β1 binds to and causes heterodimerization of TGF-β receptor type 1 (TβRI, also known as activin-like kinase (ALK) 5) and the type II receptor (TβRII), leading to the phosphorylation of SMAD2/SMAD3, which subsequently form a complex with SMAD4 and translocate into the nucleus, acting as a transcriptional factor to regulate the fibrotic gene expression (e.g., αSMA, collagen I, III or TNC). SMAD6/7 are inhibitory SMADs to inhibit transcription of SMAD2 and SMAD3. In canonical pathways, TGF-β1 can also induce SMAD-independent noncanonical signaling that involves several mitogen-activated protein kinases, including extracellular signal-regulated kinase (Erk), c-Jun-N-terminal kinase (JNK), TGF-β-activated kinase 1 (TAK1), Rho family of small GTPase, and p38 MAPK pathways.

through AT receptor expression. For instance, pro-inflammatory mediators (e.g., NF-κβ, IL-1β, IL-6, and TNF-α) make fibroblasts more reactive to Ang II by inducing AT1 synthesis **(Satou et al., 2018)**.

2.3. Endothelin (ET)

Endothelin was initially characterized as a potent vasoconstrictor peptide. However, it is now well-established as a multifunctional peptide with functions extending beyond vasoconstriction to encompass a range of physiological and pathological activities e.g. development, tumor growth, immune regulation, and the development of cardiac fibrosis **(Wang et al., 2015; Dhaun and Webb, 2019)**. ET-1, the predominant isoform in humans, is thought to be secreted mainly by endothelial cells, but can also be produced by every cell type. G protein-coupled receptors (GPCRs) ETA and ETB are two recognized ET-1 receptors. Although ET-1 acts mainly through ETA to promote vasoconstriction, inflammation, and cell proliferation, the ETB receptor is considered a physiological antagonist. ET-1 exerts fibrogenic effects, acting as a downstream molecule of cytokines and neurohumoral mediators, thus linking inflammation and cardiac fibrosis **(Barton**

and Yanagisawa, 2019).

Both Ang II and TGF-β induce ET-1 expression **(Shi-wen et al., 2007; Liu et al., 2019b)** and ET-1 upregulation is consistently confirmed in many fibrosis-associated cardiac pathologies, including MI, HF, and hypertensive heart disease **(Barton and Yanagisawa, 2019)**. Both genetic models and pharmacologic inhibition studies suggest the fibrogenic effects of ET-1 in myocardial disease. Cardiac ET-1 overexpression in mice induces myocardial fibrosis associated with both systolic and diastolic dysfunction **(Mueller et al., 2011)**.

Endothelium-specific loss of ET-1 diminished fibrosis in Ang II-infused mice **(Adiarto et al., 2012)**. ET-1 inhibition improved cardiac fibrosis **(Ammarguellat et al., 2001)**. Blocking endothelin-1 (ET-1) signaling and its fibrotic effects may hold therapeutic promise. Antagonists targeting the ETA receptor and dual ETA/ETB receptors have been shown to mitigate myocardial remodeling by reducing collagen deposition and attenuating cardiac fibrosis in animal models. The results suggest that ET-1 blockade could be a potential therapeutic approach for managing cardiac fibrosis in HFpEF. **(Valero-Munoz et al., 2016)**. Although its effectiveness in animal experiments has been shown, clinical trials using ETA antagonists have not been beneficial for patients with heart failure with reduced ejection fraction (HFrEF) and HFpEF **(Barton and Yanagisawa, 2019)**.

2.4. Platelet-Derived Growth Factors (PDGF)

The PDGFs play various roles in embryonic development, tumor progression, vascular diseases, and fibrosis. PDGFs can form homo- or heterodimers and exert their effects through two receptor tyrosine kinases known as PDGFR-α and PDGFR-β. These growth factors share common structural features, including five extracellular immunoglobulin loops and a split intracellular tyrosine kinase domain. In fibrotic conditions, PDGF signaling, which can interact with TGF-β signaling, leads to cell proliferation characterized by an activated phenotype. This ultimately results in the excessive production and deposition of ECM, contributing to fibrosis **(Andrae et al., 2008)**.

Both PDGF-A, PDGF-C, and PDGF-D are implemented as potential fibrogenic PDGFs in the myocardium through straight actions and, in part, through TGF-β **(Tuuminen et al., 2009)**. PDGF-A or PDGF-D overexpression can cause cardiac fibrosis due to excess fibroblast activation **(Gallini et al., 2016)**. Although controversial, PDGF-B is also a potent fibrogenic PDGF. PDGFR-α activation is consistently involved in myocardial fibrosis. Treatment with a neutralizing antibody against PDGFR-α and PDGFR-β attenuated collagen deposition **(Zymek et al., 2006)**.

Furthermore, a more comprehensive inhibition of PDGFR through the use of the kinase inhibitor imatinib has been shown to reduce cardiac fibrosis in mouse models of myocarditis, MI, and isoproterenol infusion **(Leipner et al., 2008; Liu et al., 2014; Wang et al., 2017)**. PDGFR-β activation potentially occurs through integrin β1 and small proline-rich repeat 3 to enhance fibroblast proliferation and matrix synthesis in a cardiac pressure overload mouse model **(Saraswati et al., 2020)**. PDGFs have also been shown to be involved in the cardiac fibrotic response in an Ang II-treated mouse model **(Nishioka et al., 2007)**.

2.5. Wnt Signaling

The Wnt signaling pathway has diverse roles in many biological processes, including carcinogenesis, embryonic development, immune maintenance, and fibrosis **(Tao et al., 2016; Zhang and Wang, 2020)**. Several reports have indicated essential roles for Wnt signaling in cardiac fibrosis progression, mainly through the TGF-β pathway. The canonical Wnt/β-catenin pathway is predominantly involved in cardiac fibrosis progression, interacting with SMAD-dependent canonical TGF-β signaling **(Xu et al., 2017; Burgy and Königshoff, 2018)**. In the absence of Wnt ligands, cytosolic β-catenin is degraded by the destruction complex, which includes tumor suppressors Axin, adenomatous polyposis coli (APC), the serine/threonine kinases, glycogen synthase kinase (GSK)-3β, casein kinase (CK) 1, protein phosphatase 2A (PP2A), and the E3-ubiquitin ligase β-transducin repeat-containing protein (β-TrCP) **(Wang et al., 2021)**.

In CFs from MI mice and human, phosphorylated GSK-3β negatively regulates TGF-β signaling by directly interacting with SMAD3 and through β-catenin signaling. Moreover, GSK-3β deletion or inhibition in in vivo models leads to hyperactivation of TGF-β-SMAD3 signaling and cardiac fibrosis **(Guo et al., 2017)**. Secreted Fz-related proteins (sFRPs), which are endogenous modulators of Wnt signaling, have emerged as key regulators of the fibrotic response. sFRP1 inhibits Wnt ligands. sFRP1 null mice show cardiac

dilation with increased expression of canonical Wnts, β-catenin, and Wnt target genes, such as Lef1 and Wisp1, leading to increased α-SMA expression and collagen production **(Huang and Huang, 2020)**. Non TGFβ signaling pathways are presented in **Figure 2**.

3. Therapeutic Options for Management of Cardiac Fibrosis

Currently, there are no drugs specifically approved for clinical use with a primary focus on anti-fibrotic actions in the context of cardiac fibrosis **(Raziyeva et al., 2022)**. While experimental studies have yielded promising results, the clinical evidence supporting the efficacy of such drugs remains limited. Despite the encouraging findings in preclinical research, the translation of anti-fibrotic therapies for cardiac fibrosis into approved clinical treatments is an ongoing challenge. Further research and clinical trials are needed to establish the safety and effectiveness of potential anti-fibrotic therapies for patients with cardiac fibrosis **(Morfino et al., 2022)**. Different agents integrated in management of cardiac fibrosis are summarized as follow:

3.1. RAAS Inhibitors

It was previously mentioned that Ang II binding to Ang II type 1 receptors (AT1R) promotes collagen synthesis, and that in chronic heart disease, there is generally a significant activation of RAAS, which is directly associated with the development of cardiac fibrosis **(Schnee, 2000)**. Various studies have shown that both angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) as seem in **Figure 3** significantly reduce myocardial fibrosis regardless of their hypotensive effect **(Brilla et al., 2000; Díez et al., 2002; Shimada et al., 2013)**.

Aldosterone, whose production is stimulated by Ang II, also exerts a pro-fibrotic effect in the myocardium by interacting with mineralocorticoid receptors **(Brilla et al., 1994)**. Aldosterone receptor antagonists (spironolactone, canrenone, and eplerenone) showed significant anti-fibrotic effects **(Mak et al., 2009; Deswal et al., 2011; Kosmala et al., 2011)**. In the RALES study (Randomized Aldactone Evaluation Study), conducted in patients with HF with reduced EF (HFrEF), spironolactone

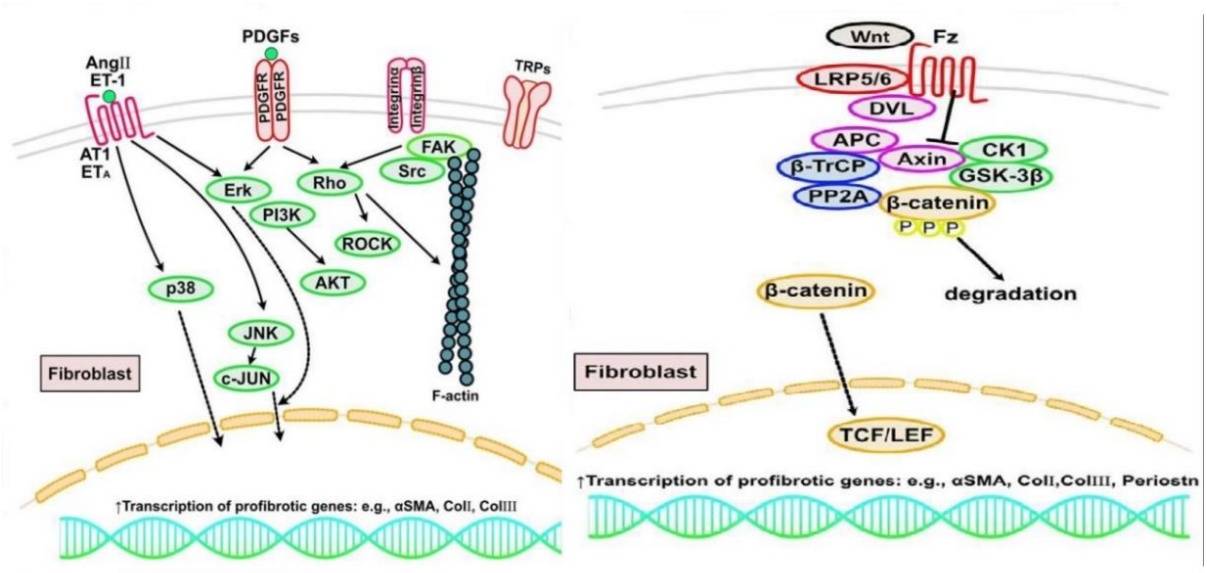

Figure 2: Non TGF-β signaling in cardiac fibrosis **(Maruyama and Imanaka-Yoshida, 2022)**. Platelet-derived growth factors (PDGFs), angiotensin (Ang) II, endothelin (ET)-1, and mechanosensitive pathways mediated by integrins and ion channels such as transient receptor potential cation channels (TRPs) can activate fibroblasts into myofibroblasts, leading to excess extracellular matrix protein deposition and cardiac fibrosis. AT1, angiotensin type 1 receptor; PDGFR, PDGF receptor; ERK, extracellular signal regulated kinase; PI3K, phosphoinositide 3-kinase; JNK, c-JUN N-terminal kinase; αSMA, α-smooth muscle actin; ROCK, Rho-associated protein kinases; FAK, focal adhesion kinase. cytosolic β-catenin is degraded by the destruction complex, which includes Axin and adenomatous polyposis coli (APC), glycogen synthase kinase (GSK)-3β and casein kinase (CK)1, protein phosphatase 2A (PP2A), and β-transducin repeat-containing protein (β-TrCP). After a Wnt ligand binds to the receptor Frizzled (Fz) and the receptor-related protein 5 or 6 (LRP5/6) coreceptor, the Wnt–Fz–LRP5/6 complex recruits Disheveled (DVL) and Axin through the intracellular domains of Fz and LRP5/6, resulting in β-catenin stabilization. The increased nuclear levels of β-catenin promote interaction with T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factor to regulate Wnt-responsive fibrotic genes.

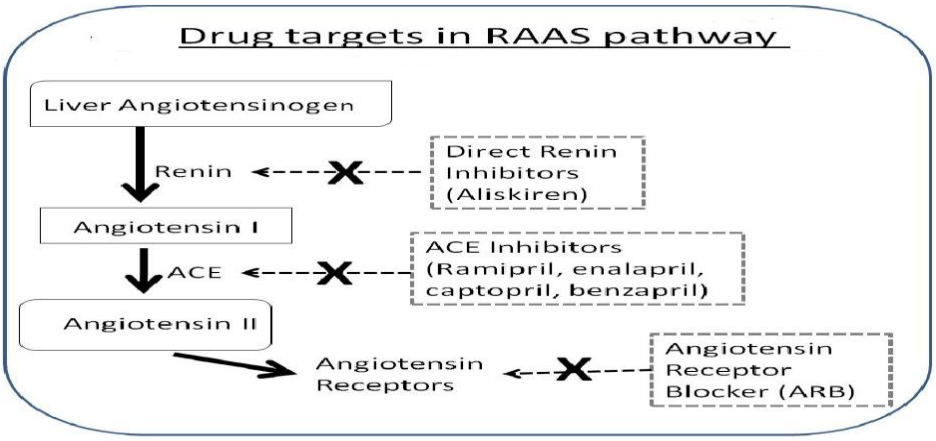

Figure 3: molecular targets in the RAAS pathway **(Zain and Awan, 2014)**.

was associated with reduced mortality and hospitalization, as well as with reduction in blood levels of fibrosis biomarkers and collagen synthesis **(Zannad et al., 2000)**.

In a sub-analysis of the study EPHESUS (the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study), which evaluated the effect of eplerenone in patients with HF after MI, treatment with eplerenone showed a significant reduction in the risk of mortality and hospitalization for all cases after 16 months in treated patients with MI complicated by subsequent LV and cardiac dysfunction compared to controls **(Iraqi et al., 2009)**. A sub-study of the ALDO-DHF trial (The Aldosterone Receptor Blockade in Diastolic Heart Failure), which included 381 patients with HFpEF, identified that treatment with spironolactone reduces PICP levels and improves diastolic function after 12 months of treatment **(Ravassa et al., 2018)**.

The administration of sacubitril/valsartan, which combines ARB and a neprilysin inhibitor, has demonstrated effectiveness in reducing fibrosis. The PARAMOUNT (Prospective Comparison of ARNI With ARB on Management of Heart Failure with Preserved Ejection Fraction) trial provided evidence that treatment with sacubitril/valsartan leads to a reduction in plasma biomarkers associated with cardiac fibrosis in patients diagnosed with HFpEF. **(Cunningham et al., 2020)**.

3.2. Inflammation Modulators

Tissue damage triggers a phlogistic process that triggers the deposition of fibrotic tissue. Tumor necrosis factor α (TNF-α) plays an important role in stimulating cardiac fibrosis **(Sun et al., 2007)**. However, the RENEWAL (Randomized etanercept Worldwide evaluation) study, which evaluated the effect of the TNF-α antagonist etanercept in patients with HF, showed no benefit in terms of mortality and hospitalization **(Mann et al., 2004)**. The ATTACH (anti-TNF Therapy Against Congestive Heart failure) study was prematurely discontinued due to increased mortality in patients with HF, a TNF-α antagonist **(Chung et al., 2003)**. The later discovery that TNF-1 and TNF-2 receptors have opposite effects on cardiac remodeling may partly explain the disappointing results of TNF-α inhibition **(Hamid et al., 2009)**.

Colchicine has an important anti-inflammatory action because of its effectiveness in inhibiting inflammasome network, various pro-inflammatory cytokines and chemokines **(Roubille et al., 2013)**. In mouse models of MI, colchicine has been shown to be effective in reducing the extent of the infarcted area. The reduction in the extent of fibrosis has been confirmed in a study on rabbit with HF **(Akodad et al., 2017)**. The COLCOT (COLCHICINE Cardiovascular Outcomes Trial) study, which randomized 4745 patients with MI to colchicine or placebo, revealed a lower risk of ischemic cardiovascular events at 30 days from MI in the treated group **(Akodad et al., 2020)**. The effect on myocardial fibrosis has not been specifically assessed. The recent COVERT-MI study (colchicine for Left ventricular Remodeling Treatment in Acute Myocardial Infarction) revealed that patients treated with colchicine after MI showed no difference in size of infarction compared to the controls **(Mewton et al., 2021)**.

Besides reducing cholesterol, statins have a powerful anti-inflammatory and cardioprotective action by inhibiting the proteins Ras, Rho, and NF-kB, and activating the PI3K/Akt/Enos pathway **(Yamamoto et al., 2011)**. Rosuvastatin has been shown to be effective in attenuating cardiac fibrosis

in mouse models of hypertensive heart disease **(Chang et al., 2009)**. Conflicting results were obtained in a sub-study of the UNIVERSE trial (The rosuvastatin Impact on VEentricular Remodeling cytokineS and neurohormonEs) **(Ashton et al., 2011)**. In conclusion, the role of statins in the treatment of chronic HF is still controversial.

3.3. Anti-TGF-β Antibodies

Transforming-β growth factor (TGF-β) has a central role in the development of cardiac fibrosis. TGF-β achieves its pro-fibrotic effect by the ALK/Smad2/3/Smad4, TAK/p-38/JNK, and NOX4/ROS signaling pathways **(Fang et al., 2017)**. In mouse models of MI and hypertensive heart disease, anti-TGF-β and ALK5 antibodies led to reduction of myocardial fibrosis but not cardiomyocyte hypertrophy. However, anti-TGF-β antibody therapy has also been associated with serious adverse effects, including LV dilation and increased mortality **(Frantz et al., 2008)**. The blockade of the TGF-β signaling pathway through antibodies therefore seems dangerous, while less intense inhibition may be more effective **(Morfino et al., 2022)**.

3.4. Pirfenidone

Pirfenidone is an oral anti-fibrotic drug approved for the treatment of idiopathic pulmonary fibrosis **(Kreuter et al., 2016)**. Due to the substantial overlap in pathophysiological mechanisms between pulmonary and cardiac fibrosis, there has been growing interest in exploring the application of pirfenidone in the treatment of cardiovascular diseases **(Aimo et al., 2022)**.

The mechanism of action of pirfenidone remains to be elucidated, but it seems to reduce the expression of pro-fibrotic factors such as TGF-β and pro-inflammatory cytokines such as TNF-α, interleukin (IL)-4, and IL-13 **(Oku et al., 2008)**. Pirfenidone also promotes MMPs expression with subsequent reduction of ECM protein accumulation **(Shi et al., 2011)**. Pirfenidone could also contribute to the modulation of activation and proliferation of T and B cells, thus regulating the secretion of numerous pro-inflammatory and pro-fibrotic molecules, such as TNF-α and TGF-β **(Du et al., 2017; Visner et al., 2009)**. Different actions of pirfenidone in management of cardiac fibrosis are illustrated in **Figure 4**.

In mouse models of hypertension, the administration of pirfenidone has been associated with reduced LV hypertrophy and increased survival compared to controls with reduction in ventricular remodeling and preventing interstitial fibrosis induced by Ang II infusion **(Yamazaki et al., 2012)**. Only two retrospective studies evaluated the efficacy of pirfenidone on cardiac parameters in patients with IPF. In both, no association was found between pirfenidone administration and cardiac functions **(Al-Ansari et al., 2020; AlAnsari et al., 2020)**.

In the PIROUETTE phase II study, which involved 94 patients with HFpEF and extended fibrosis, the use of pirfenidone led to a modest reduction in ECV. Specifically, after a 52-week follow-up, the pirfenidone-treated group experienced an absolute reduction of 0.7% in ECV, whereas the placebo-treated controls showed an increase of 0.5%. However, it's important to note that this limited effect was not associated with

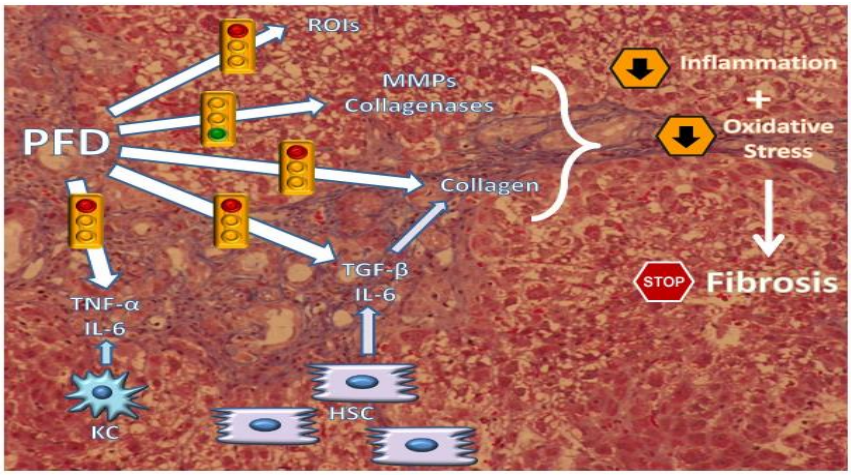

Figure 4: Molecular mechanisms of pirfenidone (PFD) in the reduction in fibrosis. Reactive oxygen species (ROS) are scavenged, transforming growth factor (TGF-β) and Interleukin 6 (IL-6) are downregulated. Inflammation markers e.g. tumor necrosis factor (TNF-α) are inhibited **(Macías-Barragán et al., 2010)**.

significant changes in diastolic function parameters **(Lewis et al., 2021)**.

4. Empagliflozin as a Future Candidate for Cardiac Fibrosis

Empagliflozin is a relatively recent medication that functions as an inhibitor of the sodium-glucose cotransporter 2 (SGLT2). Its primary purpose is to increase the excretion of glucose through urine, leading to improved glycemic control and glucose metabolism. This drug has demonstrated effectiveness in reducing glucotoxicity and insulin resistance, making it valuable for patients with type 2 diabetes mellitus (T2DM). Moreover, empagliflozin has exhibited additional benefits, such as nephroprotection, and has emerged as a significant advancement in the treatment of heart failure (HF) **(Forycka et al., 2022)**.

Empagliflozin has shown several notable benefits in clinical trials. It is associated with a reduction in heart failure-related hospitalizations and a decreased risk of cardiovascular-related deaths. Additionally, empagliflozin treatment can lower the chances of renal events, including death from kidney-related causes and the development of end-stage renal failure. The drug is generally well-tolerated and considered safe. In patients with inadequate control of blood sugar levels, empagliflozin, alone or in combination with other treatments, effectively reduces fasting and post-meal blood glucose levels, average daily glucose levels and glycated hemoglobin A1C (HbA1C). It also results in significant weight loss for individuals with T2DM **(Frampton, 2022)**.

The SGLT2 inhibitor empagliflozin was found to ameliorate myocardial fibrosis partly through inhibition of collagen formation and deposition via the classical TGF-β/Smad pathway and decreases oxidative stress via promoting Nrf2 translocation to the nucleus and activating Nrf2/ARE signalling in the type 2 diabetic KK-Ay mice model. In addition, 8 weeks of empagliflozin treatment rescues the LV structure and function in diabetic mice **(Li et al., 2019)**.

In a recent study, dapagliflozin -another SGLT2i- treatment increased cardiac ejection fraction and attenuated myocardial fibrosis in normoglycemic congestive heart failure rabbits. Dapagliflozin produced this effect through suppressing collagen formation and deposition via the classical TGF-β1/SMAD pathway which attenuated myocardial fibrosis **(Chen et al., 2022)**. The present review recommends studying such effects on empagliflozin.

5. Conclusion

Current review provided a concise summary on the current understanding of process of fibrosis in cardiac tissues, with different signaling molecules implemented in initiation, progression and establishment of such histopathological picture in myocardium. Present work also demonstrated a promising role for empagliflozin as a new anti-fibrotic effect in rats against fibrosis in the myocardium. More work is needed to elucidate molecular mechanisms and signaling pathways targeted by empagliflozin in cardiac fibrosis inhibition. This review is opening the door to the possibility of studying empagliflozin therapy for protection against cardiac fibrosis in different cardiovascular diseases in non-diabetic or pre-diabetic settings.

Declaration of competing interest

The authors declare no conflicts of interest.

References

Adiarto, S., Heiden, S., Vignon-Zellweger, N., Nakayama, K., Yagi, K., Yanagisawa, M., Emoto, N., 2012. ET-1 from endothelial cells is required for complete angiotensin II-induced cardiac fibrosis and hypertrophy. Life Sciences 91, 651–657. https://doi.org/10.1016/j.lfs.2012.02.006

Aimo, A., Spitaleri, G., Panichella, G., Lupón, J., Emdin, M., Bayes-Genis, A., 2022. Pirfenidone as a novel cardiac protective treatment. Heart Failure Reviews 27, 525–532. https://doi.org/10.1007/s10741-021-10175-w

Akodad, M., Fauconnier, J., Sicard, P., Huet, F., Blandel, F., Bourret, A., De Santa Barbara, P., Aguilhon, S., LeGall, M., Hugon, G., Lacampagne, A., Roubille, F., 2017. Interest of colchicine in the treatment of acute myocardial infarct responsible for heart failure in a mouse model. International Journal of Cardiology 240, 347–353. https://doi.org/10.1016/j.ijcard.2017.03.126

Akodad, M., Sicard, P., Fauconnier, J., Roubille, F., 2020. Colchicine and myocardial infarction: A review. Archives of Cardiovascular Diseases 113, 652–659. https://doi.org/10.1016/j.acvd.2020.04.007

Al-Ansari, S., Borowski, A., Fuad, A., Alawadhi, O., Riaz, H., Sharma, V., Khan, N., Southern, B.D., Tang, W.H.W., 2020. Effects of Pirfenidone on Echocardiographic Parameters of Left Ventricular Structure and Function in Patients with Idiopathic Pulmonary Fibrosis. Journal of Interdisciplinary Medicine 5, 35–42. https://doi.org/10.2478/jim-2020-0009

AlAnsari, S., Southern, B.D., Sharma, V., Mahalwar, G., Tang, W.W., 2020. Pirfenidone is Associated with Decreased Indexed End Diastolic and Systolic Volumes in Patients with HFpEF and a Known History of Idiopathic Pulmonary Fibrosis. Journal of Cardiac Failure 26, S25. https://doi.org/10.1016/j.cardfail.2020.09.080

Ammarguellat, F., Larouche, I., Schiffrin, E.L., 2001. Myocardial Fibrosis in DOCA-Salt Hypertensive Rats: Effect of Endothelin ET A Receptor Antagonism. Circulation 103, 319–324. https://doi.org/10.1161/01.CIR.103.2.319

Andrae, J., Gallini, R., Betsholtz, C., 2008. Role of platelet-derived growth factors in physiology and medicine. Genes & Development 22, 1276–1312. https://doi.org/10.1101/gad.1653708

Aragón, E., Wang, Q., Zou, Y., Morgani, S.M., Ruiz, L., Kaczmarska, Z., Su, J., Torner, C., Tian, L., Hu, J., Shu, W., Agrawal, S., Gomes, T., Márquez, J.A., Hadjantonakis, A.-K., Macias, M.J., Massagué, J., 2019. Structural basis for distinct roles of SMAD2 and SMAD3 in FOXH1 pioneer-directed TGF-β signaling. Genes & Development 33, 1506–1524. https://doi.org/10.1101/gad.330837.119

Ashton, E., Windebank, E., Skiba, M., Reid, C., Schneider, H., Rosenfeldt, F., Tonkin, A., Krum, H., 2011. Why did high-dose rosuvastatin not improve cardiac remodeling in chronic heart failure? Mechanistic insights from the UNIVERSE study. International Journal of Cardiology 146, 404–407. https://doi.org/10.1016/j.ijcard.2009.12.028

Barton, M., Yanagisawa, M., 2019. Endothelin: 30 Years from Discovery to Therapy. Hypertension 74, 1232–1265. https://doi.org/10.1161/HYPERTENSIONAHA.119.12105

Batlle, E., Massagué, J., 2019. Transforming Growth Factor-β Signaling in Immunity and Cancer. Immunity 50, 924–940.

Brilla, C.G., Funck, R.C., Rupp, H., 2000. Lisinopril-Mediated Regression of Myocardial Fibrosis in Patients with Hypertensive Heart Disease. Circulation 102, 1388–1393. https://doi.org/10.1161/01.CIR.102.12.1388

Brilla, C.G., Zhou, G., Matsubara, L., Weber, K.T., 1994. Collagen Metabolism in Cultured Adult Rat Cardiac Fibroblasts: Response to Angiotensin II and Aldosterone. Journal of Molecular and Cellular Cardiology 26, 809–820. https://doi.org/10.1006/jmcc.1994.1098

Brown, Marshall, 2019. Integrin-Mediated TGFβ Activation Modulates the Tumour Microenvironment. Cancers 11, 1221. https://doi.org/10.3390/cancers11091221

Burgy, O., Königshoff, M., 2018. The WNT signaling pathways in wound healing and fibrosis. Matrix Biology 68–69, 67–80. https://doi.org/10.1016/j.matbio.2018.03.017

Cavalera, M., Wang, J., Frangogiannis, N.G., 2014. Obesity, metabolic dysfunction, and cardiac fibrosis: pathophysiological pathways, molecular mechanisms, and therapeutic opportunities. Translational Research 164, 323–335. https://doi.org/10.1016/j.trsl.2014.05.001

Chang, S.-A., Kim, Y.-J., Lee, H.-W., Kim, D.-H., Kim, H.-K., Chang, H.-J., Sohn, D.-W., Oh, B.-H., Park, Y.-B., 2009. Effect of Rosuvastatin on Cardiac Remodeling, Function, and Progression to Heart Failure in Hypertensive Heart with Established Left Ventricular Hypertrophy. Hypertension 54, 591–597. https://doi.org/10.1161/HYPERTENSIONAHA.109.131243

Chen, X., Yang, Q., Bai, W., Yao, W., Liu, L., Xing, Y., Meng, C., Qi, P., Dang, Y., Qi, X., 2022. Dapagliflozin Attenuates Myocardial Fibrosis by Inhibiting the TGF-β1/Smad Signaling Pathway in a Normoglycemic Rabbit Model of Chronic Heart Failure. Frontiers of Pharmacology 13, 873108. https://doi.org/10.3389/fphar.2022.873108

Chothani, S., Schäfer, S., Adami, E., Viswanathan, S., Widjaja, A.A., Langley, S.R., Tan, J., Wang, M., Quaife, N.M., Jian Pua, C., D'Agostino, G., Guna Shekeran, S., George, B.L., Lim, S., Yiqun Cao, E., Van Heesch, S., Witte, F., Felkin, L.E., Christodoulou, E.G., Dong, J., Blachut, S., Patone, G., Barton, P.J.R., Hubner, N., Cook, S.A., Rackham, O.J.L., 2019. Widespread Translational

https://doi.org/10.1016/j.immuni.2019.03.024

Binding Proteins. Circulation 140, 937–951. https://doi.org/10.1161/CIRCULATIONAHA.119.039596

Chung, E.S., Packer, M., Lo, K.H., Fasanmade, A.A., Willerson, J.T., 2003. Randomized, Double-Blind, Placebo-Controlled, Pilot Trial of Infliximab, a Chimeric Monoclonal Antibody to Tumor Necrosis Factor-α, in Patients With Moderate-to-Severe Heart Failure: Results of the Anti-TNF Therapy Against Congestive Heart failure (ATTACH) Trial. Circulation 107, 3133–3140. https://doi.org/10.1161/01.CIR.0000077913.60364.D2

Chung, J.Y.-F., Chan, M.K.-K., Li, J.S.-F., Chan, A.S.-W., Tang, P.C.-T., Leung, K.-T., To, K.-F., Lan, H.-Y., Tang, P.M.-K., 2021. TGF-β Signaling: From Tissue Fibrosis to Tumor Microenvironment. International Journal of Molecular Sciences 22, 7575. https://doi.org/10.3390/ijms22147575

Cunningham, J.W., Claggett, B.L., O'Meara, E., Prescott, M.F., Pfeffer, M.A., Shah, S.J., Redfield, M.M., Zannad, F., Chiang, L.-M., Rizkala, A.R., Shi, V.C., Lefkowitz, M.P., Rouleau, J., McMurray, J.J.V., Solomon, S.D., Zile, M.R., 2020. Effect of Sacubitril/Valsartan on Biomarkers of Extracellular Matrix Regulation in Patients with HFpEF. Journal of the American College of Cardiology 76, 503–514. https://doi.org/10.1016/j.jacc.2020.05.072

Deswal, A., Richardson, P., Bozkurt, B., Mann, D.L., 2011. Results of the Randomized Aldosterone Antagonism in Heart Failure with Preserved Ejection Fraction Trial (RAAM-PEF). Journal of Cardiac Failure 17, 634–642. https://doi.org/10.1016/j.cardfail.2011.04.007

Dhaun, N., Webb, D.J., 2019. Endothelins in cardiovascular biology and therapeutics. Nature Reviews. Cardiology 16, 491–502. https://doi.org/10.1038/s41569-019-0176-3

Díez, J., Querejeta, R., López, B., González, A., Larman, M., Martínez Ubago, J.L., 2002. Losartan-Dependent Regression of Myocardial Fibrosis Is Associated with Reduction of Left Ventricular Chamber Stiffness in Hypertensive Patients. Circulation 105, 2512–2517. https://doi.org/10.1161/01.CIR.0000017264.66561.3D

Du, J., Paz, K., Flynn, R., Vulic, A., Robinson, T.M., Lineburg, K.E., Alexander, K.A., Meng, J., Roy, S., Panoskaltsis-Mortari, A., Loschi, M., Hill,G.R., Serody, J.S., Maillard, I., Miklos, D., Control of Fibrosis in the Human Heart by RNA-

J., Cutler, C.S., Antin, J.H., Ritz, J., MacDonald, K.P., Schacker, T.W., Luznik, L., Blazar, B.R., 2017. Pirfenidone ameliorates murine chronic GVHD through inhibition of macrophage infiltration and TGF-β production. Blood 129, 2570–2580. https://doi.org/10.1182/blood-2017-01-758854

Fang, L., Murphy, A.J., Dart, A.M., 2017. A Clinical Perspective of Anti-Fibrotic Therapies for Cardiovascular Disease. Frontiers of Pharmacology 8. https://doi.org/10.3389/fphar.2017.00186

Finnson, K.W., Almadani, Y., Philip, A., 2020. Non-canonical (non-SMAD2/3) TGF-β signaling in fibrosis: Mechanisms and targets. Seminars in Cell & Developmental Biology 101, 115–122. https://doi.org/10.1016/j.semcdb.2019.11.013

Forrester, S.J., Booz, G.W., Sigmund, C.D., Coffman, T.M., Kawai, T., Rizzo, V., Scalia, R., Eguchi, S., 2018. Angiotensin II Signal Transduction: An Update on Mechanisms of Physiology and Pathophysiology. Physiological Reviews 98, 1627–1738. https://doi.org/10.1152/physrev.00038.2017

Forycka, J., Hajdys, J., Krzemińska, J., Wilczopolski, P., Wronka, M., Młynarska, E., Rysz, J., Franczyk, B., 2022. New Insights into the Use of Empagliflozin—A Comprehensive Review. Biomedicines 10, 3294. https://doi.org/10.3390/biomedicines10123294

Frampton, J.E., 2022. Empagliflozin: A Review in Symptomatic Chronic Heart Failure. Drugs 82, 1591–1602. https://doi.org/10.1007/s40265-022-01778-0

Frangogiannis, N.G., 2021. Cardiac fibrosis. Cardiovascular Research 117, 1450–1488. https://doi.org/10.1093/cvr/cvaa324

Frangogiannis, N.G., 2020. Transforming growth factor–β in tissue fibrosis. Journal of Experimental Medicine 217, e20190103. https://doi.org/10.1084/jem.20190103

Frantz, S., Hu, K., Adamek, A., Wolf, J., Sallam, A., Kg Maier, S., Lonning, S., Ling, H., Ertl, G., Bauersachs, J., 2008. Transforming growth factor beta inhibition increases mortality and left ventricular dilatation after myocardial infarction. Basic Res Cardiol 103, 485–492. https://doi.org/10.1007/s00395-008-0739-7

Koreth,

induces cardiac fibrosis in transgenic mice. Experimental Cell Research 349, 282–290. https://doi.org/10.1016/j.yexcr.2016.10.022

McCann, G.P., Burton, J.O., 2017. Imaging of Myocardial Fibrosis in Patients with End-Stage Renal Disease: Current Limitations and Future Possibilities. BioMed Research International 2017, 1–14. https://doi.org/10.1155/2017/5453606

Guo, Y., Gupte, M., Umbarkar, P., Singh, A.P., Sui, J.Y., Force, T., Lal, H., 2017. Entanglement of GSK-3β, β-catenin and TGF-β1 signaling network to regulate myocardial fibrosis. Journal of Molecular and Cellular Cardiology 110, 109–120. https://doi.org/10.1016/j.yjmcc.2017.07.011

Hamid, T., Gu, Y., Ortines, R.V., Bhattacharya, C., Wang, G., Xuan, Y.-T., Prabhu, S.D., 2009. Divergent Tumor Necrosis Factor Receptor–Related Remodeling Responses in Heart Failure: Role of Nuclear Factor-κB and Inflammatory Activation. Circulation 119, 1386–1397. https://doi.org/10.1161/CIRCULATIONAHA.108.802918

Hanna, A., Frangogiannis, N.G., 2019. The Role of the TGF-β Superfamily in Myocardial Infarction. Frontiers in Cardiovascular Medicine 6, 140. https://doi.org/10.3389/fcvm.2019.00140

Hinderer, S., Schenke-Layland, K., 2019. Cardiac fibrosis – A short review of causes and therapeutic strategies. Advanced Drug Delivery Reviews 146, 77–82. https://doi.org/10.1016/j.addr.2019.05.011

Huang, A., Huang, Y., 2020. Role of Sfrps in cardiovascular disease. Therapeutic Advances in Chronic Disease 11, 204062232090199. https://doi.org/10.1177/2040622320901990

Huang, S., Chen, B., Su, Y., Alex, L., Humeres, C., Shinde, A.V., Conway, S.J., Frangogiannis, N.G., 2019. Distinct roles of myofibroblast-specific Smad2 and Smad3 signaling in repair and remodeling of the infarcted heart. Journal of Molecular and Cellular Cardiology 132, 84–97. https://doi.org/10.1016/j.yjmcc.2019.05.006

Imanaka-Yoshida, K., 2021. Tenascin-C in Heart Diseases—The Role of Inflammation. International Journal of Molecular Medicine 22, 5828. https://doi.org/10.3390/ijms22115828

Iraqi, W., Rossignol, P., Angioi, M., Fay, R., Nuée, J., Ketelslegers, J.M., Vincent, J., Pitt, B., Zannad, Gallini, R., Lindblom, P., Bondjers, C., Betsholtz, C., Andrae, J., 2016. PDGF-A and PDGF-B Complicated by Left Ventricular Dysfunction and Heart Failure: Insights from the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) Study. Circulation 119, 2471–2479. https://doi.org/10.1161/CIRCULATIONAHA.108.809194

Jellis, C., Martin, J., Narula, J., Marwick, T.H., 2010. Assessment of Nonischemic Myocardial Fibrosis. Journal of the American College of Cardiology 56, 89–97. https://doi.org/10.1016/j.jacc.2010.02.047

Jia, G., Aroor, A.R., Hill, M.A., Sowers, J.R., 2018. Role of Renin-Angiotensin-Aldosterone System Activation in Promoting Cardiovascular Fibrosis and Stiffness. Hypertension 72, 537–548. https://doi.org/10.1161/HYPERTENSIONAHA.118.11065

Khalil, H., Kanisicak, O., Prasad, V., Correll, R.N., Fu, X., Schips, T., Vagnozzi, R.J., Liu, R., Huynh, T., Lee, S.-J., Karch, J., Molkentin, J.D., 2017. Fibroblast-specific TGF-β–Smad2/3 signaling underlies cardiac fibrosis. Journal of Clinical Investigation 127, 3770–3783. https://doi.org/10.1172/JCI94753

Kosmala, W., Przewlocka-Kosmala, M., Szczepanik-Osadnik, H., Mysiak, A., O'Moore-Sullivan, T., Marwick, T.H., 2011. A Randomized Study of the Beneficial Effects of Aldosterone Antagonism on LV Function, Structure, and Fibrosis Markers in Metabolic Syndrome. JACC: Cardiovascular Imaging 4, 1239–1249. https://doi.org/10.1016/j.jcmg.2011.08.014

Krejci, J., Mlejnek, D., Sochorova, D., Nemec, P., 2016. Inflammatory Cardiomyopathy: A Current View on the Pathophysiology, Diagnosis, and Treatment. BioMed Research International 2016, 1–11. https://doi.org/10.1155/2016/4087632

Kreuter, M., Wuyts, W., Renzoni, E., Koschel, D., Maher, T.M., Kolb, M., Weycker, D., Spagnolo, P., Kirchgaessler, K.-U., Herth, F.J.F., Costabel, U., 2016. Antacid therapy and disease outcomes in idiopathic pulmonary fibrosis: a pooled analysis. The Lancet Respiratory Medicine 4, 381–389. https://doi.org/10.1016/S2213-2600(16)00067-9

Leipner, C., Grün, K., Müller, A., Buchdunger, E., Borsi, L., Kosmehl, H., Berndt, A., Janik, T., Uecker, A., Kiehntopf, M., Böhmer, F.-D., 2008.

F., 2009. Extracellular Cardiac Matrix Biomarkers in Patients with Acute Myocardial Infarction

Cardiovascular Research 79, 118–126. https://doi.org/10.1093/cvr/cvn063

Lewis, G.A., Dodd, S., Clayton, D., Bedson, E., Eccleson, H., Schelbert, E.B., Naish, J.H., Jimenez, B.D., Williams, S.G., Cunnington, C., Ahmed, F.Z., Cooper, A., Rajavarma Viswesvaraiah, Russell, S., McDonagh, T., Williamson, P.R., Miller, C.A., 2021. Pirfenidone in heart failure with preserved ejection fraction: a randomized phase 2 trial. Nat Med 27, 1477–1482. https://doi.org/10.1038/s41591-021-01452-0

Li, C., Zhang, J., Xue, M., Li, X., Han, F., Liu, X., Xu, L., Lu, Y., Cheng, Y., Li, T., Yu, X., Sun, B., Chen, L., 2019. SGLT2 inhibition with empagliflozin attenuates myocardial oxidative stress and fibrosis in diabetic mice heart. Cardiovascular Diabetology 18, 15. https://doi.org/10.1186/s12933-019-0816-2

Li, L., Zhao, Q., Kong, W., 2018. Extracellular matrix remodeling and cardiac fibrosis. Matrix Biology 68–69, 490–506. https://doi.org/10.1016/j.matbio.2018.01.013

Liu, C., Lim, S.T., Teo, M.H.Y., Tan, M.S.Y., Kulkarni, M.D., Qiu, B., Li, A., Lal, S., Dos Remedios, C.G., Tan, N.S., Wahli, W., Ferenczi, M.A., Song, W., Hong, W., Wang, X., 2019a. Collaborative Regulation of LRG1 by TGF-β1 and PPAR-β/δ Modulates Chronic Pressure Overload–Induced Cardiac Fibrosis. Circulation. Heart Failure 12, e005962. https://doi.org/10.1161/CIRCHEARTFAILURE.119.005962

Liu, C., Zhao, W., Meng, W., Zhao, T., Chen, Y., Ahokas, R.A., Liu, H., Sun, Y., 2014. Platelet-derived growth factor blockade on cardiac remodeling following infarction. Molecular and Cellular Biochemistry 397, 295–304. https://doi.org/10.1007/s11010-014-2197-x

Liu, G., Ma, C., Yang, H., Zhang, P.-Y., 2017. Transforming growth factor β and its role in heart disease. Experimental and Therapeutic Medicine 13, 2123–2128. https://doi.org/10.3892/etm.2017.4246

Liu, J., Zhuang, T., Pi, J., Chen, X., Zhang, Q., Li, Y., Wang, H., Shen, Y., Tomlinson, B., Chan, P., Yu, Z., Cheng, Y., Zheng, X., Reilly, M., Morrisey, E., Zhang, L., Liu, Z., Zhang, Y., 2019b. Endothelial Forkhead Box Transcription Factor P1 Regulates Imatinib mesylate attenuates fibrosis in coxsackievirus b3-induced chronic myocarditis.

Endothelin-1 Signal Pathway. Circulation 140, 665–680. https://doi.org/10.1161/CIRCULATIONAHA.119.039767

Macías-Barragán, J., Sandoval-Rodríguez, A., Navarro-Partida, J., Armendáriz-Borunda, J., 2010. The multifaceted role of pirfenidone and its novel targets. Fibrogenesis Tissue Repair 3, 16. https://doi.org/10.1186/1755-1536-3-16

Mak, G.J., Ledwidge, M.T., Watson, C.J., Phelan, D.M., Dawkins, I.R., Murphy, N.F., Patle, A.K., Baugh, J.A., McDonald, K.M., 2009. Natural History of Markers of Collagen Turnover in Patients With Early Diastolic Dysfunction and Impact of Eplerenone. Journal of the American College of Cardiology 54, 1674–1682. https://doi.org/10.1016/j.jacc.2009.08.021

Mann, D.L., McMurray, J.J.V., Packer, M., Swedberg, K., Borer, J.S., Colucci, W.S., Djian, J., Drexler, H., Feldman, A., Kober, L., Krum, H., Liu, P., Nieminen, M., Tavazzi, L., Van Veldhuisen, D.J., Waldenstrom, A., Warren, M., Westheim, A., Zannad, F., Fleming, T., 2004. Targeted Anticytokine Therapy in Patients With Chronic Heart Failure: Results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). Circulation 109, 1594–1602. https://doi.org/10.1161/01.CIR.0000124490.27666.B2

Maruyama, K., Imanaka-Yoshida, K., 2022. The Pathogenesis of Cardiac Fibrosis: A Review of Recent Progress. International Journal of Molecular Sciences 23, 2617. https://doi.org/10.3390/ijms23052617

Mewton, N., Roubille, F., Bresson, D., Prieur, C., Bouleti, C., Bochaton, T., Ivanes, F., Dubreuil, O., Biere, L., Hayek, A., Derimay, F., Akodad, M., Alos, B., Haider, L., El Jonhy, N., Daw, R., De Bourguignon, C., Dhelens, C., Finet, G., Bonnefoy-Cudraz, E., Bidaux, G., Boutitie, F., Maucort-Boulch, D., Croisille, P., Rioufol, G., Prunier, F., Angoulvant, D., on behalf of the COVERT-MI Study Investigators, 2021. Effect of Colchicine on Myocardial Injury in Acute Myocardial Infarction. Circulation 144, 859–869. https://doi.org/10.1161/CIRCULATIONAHA.121.056177

Micheletti, R., Plaisance, I., Abraham, B.J., Sarre,

Pathological Cardiac RemodelingThrough Transforming Growth Factor-β1–

The long noncoding RNA Wisper controls cardiac fibrosis and remodeling. Science Translational Medicine 9, eaai9118. https://doi.org/10.1126/scitranslmed.aai9118

Morfino, P., Aimo, A., Castiglione, V., Gálvez-Montón, C., Emdin, M., Bayes-Genis, A., 2022. Treatment of cardiac fibrosis: from neuro-hormonal inhibitors to CAR-T cell therapy. Heart Failure Reviews https://doi.org/10.1007/s10741-022-10279-x

Mueller, E.E., Momen, A., Massé, S., Zhou, Y.-Q., Liu, J., Backx, P.H., Henkelman, R.M., Nanthakumar, K., Stewart, D.J., Husain, M., 2011. Electrical remodelling precedes heart failure in an endothelin-1-induced model of cardiomyopathy. Cardiovascular Research 89, 623–633. https://doi.org/10.1093/cvr/cvq351

Nicin, L., Wagner, J.U.G., Luxán, G., Dimmeler, S., 2022. Fibroblast-mediated intercellular crosstalk in the healthy and diseased heart. FEBS Letters 596, 638–654. https://doi.org/10.1002/1873-3468.14234

Nishioka, T., Suzuki, M., Onishi, K., Takakura, N., Inada, H., Yoshida, T., Hiroe, M., Imanaka-Yoshida, K., 2007. Eplerenone Attenuates Myocardial Fibrosis in the Angiotensin II-Induced Hypertensive Mouse: Involvement of Tenascin-C Induced by Aldosterone-Mediated Inflammation. Journal of Cardiovascular Pharmacology 49, 261–268. https://doi.org/10.1097/FJC.0b013e318033dfd4

Oku, H., Shimizu, T., Kawabata, T., Nagira, M., Hikita, I., Ueyama, A., Matsushima, S., Torii, M., Arimura, A., 2008. Antifibrotic action of pirfenidone and prednisolone: Different effects on pulmonary cytokines and growth factors in bleomycin-induced murine pulmonary fibrosis. European Journal of Pharmacology 590, 400–408. https://doi.org/10.1016/j.ejphar.2008.06.046

Paz Ocaranza, M., Riquelme, J.A., García, L., Jalil, J.E., Chiong, M., Santos, R.A.S., Lavandero, S., 2020. Counter-regulatory renin–angiotensin system in cardiovascular disease. Nature Reviews. Cardiology 17, 116–129. https://doi.org/10.1038/s41569-019-0244-8

Ravassa, S., Trippel, T., Bach, D., Bachran, D., González, A., López, B., Wachter, R., Hasenfuss, G., Delles, C., Dominiczak, A.F., Pieske, B., Díez, A., Ting, C.-C., Alexanian, M., Maric, D., Maison, D., Nemir, M., Young, R.A., Schroen, B., González, A., Ounzain, S., Pedrazzini, T., 2017. fraction resistant to the beneficial effects of spironolactone: results from the Aldo-DHF trial: Myocardial fibrosis and effect of spironolactone in HFpEF. European Journal of Heart Failure 20, 1290–1299. https://doi.org/10.1002/ejhf.1194

Raziyeva, K., Kim, Y., Zharkinbekov, Z., Temirkhanova, K., Saparov, A., 2022. Novel Therapies for the Treatment of Cardiac Fibrosis Following Myocardial Infarction. Biomedicines 10, 2178. https://doi.org/10.3390/biomedicines10092178

Robertson, I.B., Rifkin, D.B., 2016. Regulation of the Bioavailability of TGF-β and TGF-β-Related Proteins. Cold Spring Harbor Perspectives in Biology 8, a021907. https://doi.org/10.1101/cshperspect.a021907

Roubille, F., Kritikou, E., Busseuil, D., Barrere-Lemaire, S., Tardif, J.-C., 2013. Colchicine: An Old Wine in a New Bottle? Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry 12, 14–23. https://doi.org/10.2174/1871523011312010004

Sainio, A., Järveläinen, H., 2020. Extracellular matrix-cell interactions: Focus on therapeutic applications. Cellular Signalling 66, 109487. https://doi.org/10.1016/j.cellsig.2019.109487

Saraswati, S., Lietman, C.D., Li, B., Mathew, S., Zent, R., Young, P.P., 2020. Small proline-rich repeat 3 is a novel coordinator of PDGFRβ and integrin β1 crosstalk to augment proliferation and matrix synthesis by cardiac fibroblasts. FASEB journal 34, 7885–7904. https://doi.org/10.1096/fj.201902815R

Satou, R., Penrose, H., Navar, L.G., 2018. Inflammation as a Regulator of the Renin-Angiotensin System and Blood Pressure. Current Hypertension Reports 20, 100. https://doi.org/10.1007/s11906-018-0900-0

Schiller, M., Javelaud, D., Mauviel, A., 2004. TGF-β-induced SMAD signaling and gene regulation: consequences for extracellular matrix remodeling and wound healing. Journal of Dermatological Science 35, 83–92. https://doi.org/10.1016/j.jdermsci.2003.12.006

Schnee, J., 2000. Angiotensin II, adhesion, and

J., Edelmann, F., 2018. Biomarker-based phenotyping of myocardial fibrosis identifies patients with heart failure with preserved ejection

Shi, Q., Liu, X., Bai, Y., Cui, C., Li, J., Li, Y., Hu, S., Wei, Y., 2011. In Vitro Effects of Pirfenidone on Cardiac Fibroblasts: Proliferation, Myofibroblast Differentiation, Migration and Cytokine Secretion. PLoS ONE 6, e28134. https://doi.org/10.1371/journal.pone.0028134

Shimada, Y.J., Passeri, J.J., Baggish, A.L., O'Callaghan, C., Lowry, P.A., Yannekis, G., Abbara, S., Ghoshhajra, B.B., Rothman, R.D., Ho, C.Y., Januzzi, J.L., Seidman, C.E., Fifer, M.A., 2013. Effects of Losartan on Left Ventricular Hypertrophy and Fibrosis in Patients with Nonobstructive Hypertrophic Cardiomyopathy. JACC: Heart Failure 1, 480–487. https://doi.org/10.1016/j.jchf.2013.09.001

Shi-wen, X., Kennedy, L., Renzoni, E.A., Bou-Gharios, G., Du Bois, R.M., Black, C.M., Denton, C.P., Abraham, D.J., Leask, A., 2007. Endothelin is a downstream mediator of profibrotic responses to transforming growth factor β in human lung fibroblasts. Arthritis & Rheumatism 56, 4189–4194. https://doi.org/10.1002/art.23134

Sun, M., Chen, M., Dawood, F, Zurawska, U., Li, J.Y., Parker, T., Kassiri, Z., Kirshenbaum, L.A., Arnold, M., Khokha, R., Liu, P.P., 2007. Tumor Necrosis Factor-α Mediates Cardiac Remodeling and Ventricular Dysfunction After Pressure Overload State. Circulation 115, 1398–1407. https://doi.org/10.1161/CIRCULATIONAHA.106.643585

Tao, H., Yang, J.-J., Shi, K.-H., Li, J., 2016. Wnt signaling pathway in cardiac fibrosis: New insights and directions. Metabolism 65, 30–40. https://doi.org/10.1016/j.metabol.2015.10.013

Tuuminen, R., Nykänen, A.I., Krebs, R., Soronen, J., Pajusola, K., Keränen, M.A.I., Koskinen, P.K., Alitalo, K., Lemström, K.B., 2009. PDGF-A, -C, and -D but not PDGF-B Increase TGF-β1 and Chronic Rejection in Rat Cardiac Allografts. Arteriosclerosis, Thrombosis, and Vascular Biology 29, 691–698. https://doi.org/10.1161/ATVBAHA.108.178558

Tzavlaki, K., Moustakas, A., 2020. TGF-β Signaling. Biomolecules 10, 487. https://doi.org/10.3390/biom10030487

Valero-Munoz, M., Li, S., Wilson, R.M., Boldbaatar, B., Iglarz, M., Sam, F., 2016. Dual Endothelin-cardiac fibrosis. Cardiovascular Research 46, 264–268. https://doi.org/10.1016/S0008-6363(00)00044-4

Failure 9, e003381. https://doi.org/10.1161/CIRCHEARTFAILURE.116.003381

Visner, G.A., Liu, F., Bizargity, P., Liu, H., Liu, K., Yang, J., Wang, L., Hancock, W.W., 2009. Pirfenidone Inhibits T-Cell Activation, Proliferation, Cytokine and Chemokine Production, and Host Alloresponses. Transplantation 88, 330–338. https://doi.org/10.1097/TP.0b013e3181ae3392

Wang, L.-X., Yang, X., Yue, Y., Fan, T., Hou, J., Chen, G.-X., Liang, M.-Y., Wu, Z.-K., 2017. Imatinib attenuates cardiac fibrosis by inhibiting platelet-derived growth factor receptors activation in isoproterenol induced model. PLoS ONE 12, e0178619. https://doi.org/10.1371/journal.pone.0178619

Wang, X., Guo, Z., Ding, Z., Khaidakov, M., Lin, J., Xu, Z., Sharma, S.G., Jiwani, S., Mehta, J.L., 2015. Endothelin-1 upregulation mediates aging-related cardiac fibrosis. Journal of Molecular and Cellular Cardiology 80, 101–109. https://doi.org/10.1016/j.yjmcc.2015.01.001

Wang, Z., Zhao, T., Zhang, S., Wang, J., Chen, Y., Zhao, H., Yang, Y., Shi, S., Chen, Q., Liu, K., 2021. The Wnt signaling pathway in tumorigenesis, pharmacological targets, and drug development for cancer therapy. Biomarkers Research 9, 68. https://doi.org/10.1186/s40364-021-00323-7

Xu, L., Cui, W.-H., Zhou, W.-C., Li, D.-L., Li, L.-C., Zhao, P., Mo, X.-T., Zhang, Z., Gao, J., 2017. Activation of Wnt/β-catenin signalling is required for TGF-β/Smad2/3 signalling during myofibroblast proliferation. Journal of Cellular and Molecular Medicine 21, 1545–1554. https://doi.org/10.1111/jcmm.13085

Yamamoto, C., Fukuda, N., Jumabay, M., Saito, K., Matsumoto, T., Ueno, T., Soma, M., Matsumoto, K., Shimosawa, T., 2011. Protective effects of statin on cardiac fibrosis and apoptosis in adrenomedullin-knockout mice treated with angiotensin II and high salt loading. Hypertension Research 34, 348–353. https://doi.org/10.1038/hr.2010.243

Yamazaki, T., Yamashita, N., Izumi, Y., Nakamura, Y., Shiota, M., Hanatani, A., Shimada, K., Muro, T., Iwao, H., Yoshiyama, M., 2012. The antifibrotic agent pirfenidone inhibits angiotensin II-induced cardiac

A/Endothelin-B Receptor Blockade and Cardiac Remodeling in Heart Failure with Preserved Ejection Fraction. Circulation. Heart

Yousefi, F., Shabaninejad, Z., Vakili, S., Derakhshan, M., Movahedpour, A., Dabiri, H., Ghasemi, Y., Mahjoubin-Tehran, M., Nikoozadeh, A., Savardashtaki, A., Mirzaei, H., Hamblin, M.R., 2020. TGF-β and WNT signaling pathways in cardiac fibrosis: non-coding RNAs come into focus. Cell Communication and Signaling 18, 87. https://doi.org/10.1186/s12964-020-00555-4

Zain, M., Awan, F.R., 2014. Renin Angiotensin Aldosterone System (RAAS): its biology and drug targets for treating diabetic nephropathy. Pakistan Journal of Pharmaceutical Sciences 27, 1379–1391.

Zannad, F., Alla, F., Dousset, B., Perez, A., Pitt, B., 2000. Limitation of Excessive Extracellular Matrix Turnover May Contribute to Survival Benefit of Spironolactone Therapy in Patients with Congestive Heart Failure: Insights From the Randomized Aldactone Evaluation Study (RALES). Circulation 102, 2700–2706. https://doi.org/10.1161/01.CIR.102.22.2700

Zhang, Y., Alexander, P.B., Wang, X.-F., 2017. TGF-β Family Signaling in the Control of Cell Proliferation and Survival. Cold Spring Harbor Perspectives in Biology 9, a022145. https://doi.org/10.1101/cshperspect.a022145

hypertrophy in mice. Hypertension Research 35, 34–40. https://doi.org/10.1038/hr.2011.139

Zhang, Y., Wang, X., 2020. Targeting the Wnt/β-catenin signaling pathway in cancer. Journal of Hematology & Oncology 13, 165. https://doi.org/10.1186/s13045-020-00990-3

Zhang, Y.E., 2017. Non-Smad Signaling Pathways of the TGF-β Family. Cold Spring Harbor Perspectives in Biology 9, a022129. https://doi.org/10.1101/cshperspect.a022129

Zinski, J., Tajer, B., Mullins, M.C., 2018. TGF-β Family Signaling in Early Vertebrate Development. Cold Spring Harbor Perspectives in Biology 10, a033274. https://doi.org/10.1101/cshperspect.a033274

Zymek, P., Bujak, M., Chatila, K., Cieslak, A., Thakker, G., Entman, M.L., Frangogiannis, N.G., 2006. The Role of Platelet-Derived Growth Factor Signaling in Healing Myocardial Infarcts. Journal of the American College of Cardiology 48, 2315–2323. https://doi.org/10.1016/j.jacc.2006.07.060