Myocardial fibrosis is observed in many cardiovascular diseases including hypertension, heart failure and cardiomyopathy. Myocardial fibrosis has been proved to be reversible and treatable only under timely intervention, which makes early detection and assessment of fibrosis crucial. Aside from tissue biopsy as the gold standard for the diagnosis of myocardial fibrosis, circulating biomarkers have been adopted as noninvasive assessment of this lesion. Dysregulated collagen deposition is thought to be the major cause of myocardial fibrosis. Collagens, procollagens, TGF-β, TIMP, galectin-3, and microRNAs are thought to be indicators of myocardial fibrosis. In this review, we summarize the molecules that are frequently used as biomarkers in diagnosis of cardiac fibrosis. Mechanisms of fibrosis that they take part in are also introduced.

**Keywords:** Myocardial fibrosis; Biomarker; Collagens; TGF-β; MicroRNA

## 1 Introduction

Myocardial fibrosis (MF) is cardiac remodeling characterized by over-proliferation of cardiac fibroblasts and abnormal deposition and distribution of collagens. It contributes to many kinds of cardiovascular diseases including hypertension, chronic heart failure, hypertrophic cardiomyopathy, dilated cardiomyopathy and viral myocarditis. Myocardial fibrosis is also a potential risk factor for sudden cardiac death. There is not a comprehensive statement about the pathogenesis of MF yet, but MF is thought to be associated with angiotensin II, cytokines, oxidative stress, etc. Cardiac infarction is the most important inducement of myocardial fibrosis. Several other pathophysiologic conditions can induce cardiac remodeling as well. Aging, pressure overload caused by hypertension or aortic stenosis, and volume overload are all considered as associated with the development of severe cardiac fibrosis [1,2].

In consideration of the mutual promotive relationship between cardiovascular diseases and myocardial fibrosis, it is of great significance to make MF quickly and accurately diagnosed. Serum biomarkers have been proposed to reflect fibrosis and clinical trials have been performed to examine the usage of these biomarkers in a diagnostic or prognostic way for myocardial fibrosis. The biomarkers are often molecules that alone or in concert take an essential part in the emergence and development of fibrosis and/or are dysregulated in the pathological process of fibrosis. To understand the basic characteristics and role in myocardial fibrosis of these biomarkers is of clinical value. This review lists the most normally used biomarkers in clinical diagnosis of myocardial fibrosis, common features and mechanisms of which are described. Through retrospect of literatures, we aim to get a deeper and more integral cognition of cardiac fibrosis biomarkers and hope that a better understanding of them would be helpful in seeking effective detective or therapeutic methods [3].

## 2 Collagens

As is mentioned above, myocardial fibrosis is characterized by abnormal deposition and distribution of collagens. Collagens are the main components of extracellular matrix (ECM). Increased cardiac collagen deposition and collagen turnover play an important role in development of myocardial fibrosis. The major types of collagen in the heart are collagen type I and III. Propeptides of procollagen type I and III (PIP and PIIP) are markers of collagen synthesis and breakdown. Propeptides of procollagen and collagen levels are useful tools in detecting myocardial remodeling.

In the study of victims of idiopathic myocardial fibrosis (IMF), collagen composition was examined...
Transforming growth factor β (TGF-β) is a multifunctional cytokine that can be expressed by many types of cells. TGF-β is involved in various kinds of disease including cardiac dysfunction, myocardial fibrosis, heart failure, ventricular remodeling and cardiac hypertrophy. With respect to cardiac fibrosis, TGF-β appears to participate in all related signaling pathways. TGF-β has been proved to play a central role in stimulating fibroblasts, promoting their differentiation into myofibroblasts, as well as promotion of cardiomyocyte apoptosis and cardiac hypertrophy.

TGF-β signaling initiates at binding of TGF-β to TGF-β receptor type II (TGFβRII). TGFβRII recruits TGF-β receptor type I (TGFβRI), which activates Smads. Translocation of Smads into the nucleus is followed by transcriptional reprogramming that contributes to cardiac fibrosis. Elevated TGF-β levels have been detected in different kinds of diseases with myocardial fibrosis as a symptom and is accepted as a biomarker in diagnosis of fibrosis. As TGF-β plays such a critical role in the development and progress of myocardial fibrosis, therapeutic targeting of TGF-β signaling has been receiving increased attention in recent years. It is of importance to understand mechanisms underlying TGF-β signaling in the development of myocardial fibrosis. TGF-β levels were significantly increased in patients with dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM), in which TGF-β was correlated with phosphorylated Smad2 and collagen type I and type III, suggesting that TGF-β activation contributed to excess myocardial fibrosis in both ICM and DCM [9].

An extracellular matrix (ECM) protein, fibulin-2, is essential for TGF-β/smads signaling. Smad2 phosphorylation can only be achieved in the presence of fibulin-2, indicating fibulin-2 as a critical regulator of TGF-β in development of myocardial fibrosis [10]. In a study of cardiac infarction, depressed miR-24 expression was observed. miR-24 can inhibit TGF-β signaling through increasing binding of Smad2/3, Smad4, histone deacetylase (HDAC) 1, and decreasing binding of HDAC 3 to the PPARγ promoter in cardiac fibroblasts [12]. There is research demonstrating that reactive oxygen species (ROS) that derived from NADPH oxidase 4 (Nox4) could sensitize myocardial fibroblasts to respond to TGF-β1 via TGF-β1/Smad signaling pathway [13]. In inflammatory dilated cardiomyopathy (iDCM), in response to TGF-β signaling, Wnt proteins were rapidly secreted and Wnt/β-catenin pathway was activated, mediated by Smad-independent TGF-β-activated kinase 1 (TAK1). Inactivation of Wnt or inhibition of Wnt secretion prevented TGF-β-mediated transformation of cardiac fibroblasts into pathogenic myofibroblasts, which made Wnt protein secretion a novel downstream mechanism of TGF-β-mediated myocardial fibrosis progression [14]. There is a study showing that connective tissue growth factor (CTGF) could affect the process of hypertension-induced myocardial fibrosis by regulating TGF-β mRNA expression in cardiac tissues [15].

4 Matrix metalloproteinases

Extracellular matrix (ECM) is the main component of myocardial interstitial tissue. Abnormal deposition of ECM leads to development of myocardial fibrosis. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) regulate renewal of ECM.
MMPs can induce degradation of different kinds of proteins in ECM, which is mediated by TIMPs and other cytokines. MMPs/TIMPs ratio imbalance plays an essential role in myocardial fibrosis. TIMP1 is consistently upregulated in myocardial fibrosis and is used as a biomarker of fibrosis [3,16].

MMP-9 expression was only up-regulated in myocardial fibroblasts, not in cardiac cells [17]. In a study of 54 hypertrophic cardiomyopathy (HCM) patients, MMP-9 was associated with fibrosis and with cardiac events in women, increased MMP-2 levels were associated with lower fibrosis in women and MMP-3 levels were positively related to cardiac events. These findings suggest MMP-9 as a useful biomarker for fibrosis in female HCM patients [18]. Regulation of myocardial collagen turnover and deposition by MMP-9 is thought to be related to periostin, connective tissue growth factor (CTGF) and TGF-β/Smad signaling in cardiac ageing. MMP-9 deficiency can also result in a compensatory increase in MMP-8 [19]. In isoproterenol-induced heart failure (HF), IL-17 could increase expression of MMP-1, RANKL, and type I and III collagen in cardiac fibroblasts. IL-17 induces MMP-1 depending on the RANKL/OPG system, which makes it a harmful cytokine. Blockade of IL-17 can improve myocardial fibrosis in HF [20]. MMP-12 could induce Arg 1+ macrophage accumulation, expression of TSP1, vWF and PDGFRβ and production of profibrotic mediators including PDGFBB, TGFβ1 and pSMAD2. Together, MMP-12 plays an important role of in regulating fibrosis [21]. In vivo, in cultured cardiac fibroblasts, and in human fibrotic myocardium patients with dilated cardiomyopathy (DCM), a study showed that aside from its matrix metalloproteinase-inhibitory function, TIMP-1 mediated an association between CD63, one of its cell surface receptors, and integrin β1 on cardiac fibroblasts. Smad2/3 and β-catenin were activated, leading to collagen synthesis. In addition, Timp1 deficiency could reduce myocardial fibrosis [22].

5 Galectin-3

Galectin (Gal)-3 is a β-galactoside-binding lectin predominantly expressed by activated macrophages. It is a multifunctional matricellular protein that regulates inflammatory and fibrotic responses. Aside from its role in cell proliferation, apoptosis, differentiation, angiogenesis, and adhesion, Gal-3 is proven to promote fibroblast proliferation and transformation, mediate collagen production and be involved in pathophysiology of myocardial fibrosis.

In patients with inflammatory cardiomyopathy (iCMP) and nonischemic dilated cardiomyopathy, the level of myocardial Gal-3, but not circulating concentrations of Gal-3, was found as a possible marker for cardiac fibrosis, depending on pathogenesis of heart failure [23]. In the study of 259 blood samples and endomyocardial biopsies from heart failure patients, plasma myocardial mRNA and protein expression of Gal-3 were measured. An excess of Gal-3 was observed in patients with HF originating from hypertension compared with healthy controls [24]. 150 patients were collected in a study investigating the relationship between circulating galectin-3 levels and myocardial fibrosis in patients with nonischemic dilated cardiomyopathy (NICM). Galectin-3 value was higher in patients with left ventricular (LV) fibrosis, which supports the view that galectin-3 is related to cardiac fibrosis and remodeling in NICM [25]. In 63 patients with stable coronary artery disease (CAD), gal-3 serum level was determined. Results demonstrate that the degree of myocardial fibrosis can be reflected by elevated serum levels of gal-3 [26]. Plasma Gal-3 levels were all high in patients with pediatric Kawasaki Disease (PKD), young adults late after KD onset (AKD) and aldosterone-producing adenoma (APA) [27,28]. Galectin-3 expression could be increased by PKC activation, which leads to collagen I and fibronection accumulation in cardiomyocytes. The regulation of collagen production indicates that galectin-3 can mediate PKC-induced cardiac fibrosis in heart failure [29].

6 MicroRNAs

Micro-RNAs (miRNAs) are a class of small non-coding RNAs. Recently, miRNAs have gained growing attention as a post-transcriptional regulator in various cardiac pathologies including cardiomyocyte hypertrophy, excitation-contraction coupling and myocardial fibrosis. Elevated or decreased levels of different types of miRNAs are both observed in cardiac fibrosis. Detection of miRNAs is important for early discovery of myocardial fibrosis and targeting them has become a hotspot in researching new treatments. Aside from miRNA, circulating miRNAs are also adopted as putative biomarkers for diffuse myocardial fibrosis in HCM [30].

Cardiac miR-21 levels were elevated in patients processing rejection after heart transplantation. Overexpression of miR-21 activates a fibrotic gene program as well as promotes monocyte transition to fibrocyte. Elevated levels of miR-21 were observed in patients with aortic stenosis (AS). PDCD4, RECK and effectors of TGF-β signaling are down-stream targets of miR-21. MiR-21 targets...
transforming growth factor beta receptor III (TGFβRIII), a TGF-β pathway negative regulator. TGF-β1 and miR-21 were up-regulated in response to myocardial infarction. Overexpression of miR-21 induces reduction of TGFβRIII expression and increased collagen content. While up-regulation of TGFβRIII in return inhibits the expression of miR-21, indicating a reciprocal loop between TGFβRIII and miR-21 in cardiac fibrosis. In atrial fibrillation (AF), TGF-β1 induces miR-21 up-regulation. Through specific degradation of Smad7, miR-21 increases the expression of collagen I/III in fibroblasts [31–34].

MiR-34a was upregulated in infarcted heart. TGF-β1 can increase miR-34a expression in cardiac fibroblasts while this overexpression of miR-34a would in return increase the profibrogenic activity of TGF-β1. By directly targeting Smad4, miR-34a plays a key role in the progression of cardiac fibrosis. These findings suggest that miR-34a may be a new marker for cardiac fibrosis [35].

Decreased miR-29c together with increased NOX2 and TIMP1 are involved in cardiac fibrosis development. A study of patients demonstrated that a significant amount of miRNAs would be released in cardiac remodeling associated with HCM. In chronic kidney disease (CKD), microRNA (miR)-29b-3p directly targets mRNA of collagen. Expression level of miR-29b-3p decreases in cardiac fibroblasts, which is regulated by Na/K-ATPase [36–38].

miR-328, significantly upregulated in the margin of infarcted myocardium, stimulates TGF-β1 and promotes collagen production in fibroblasts. Further study showed that the fibrotic-promotive effect miR-328 demonstrated was regulated by targeting TGFβRIII, which suggests miR-328 as an important mediator of cardiac fibrosis in diseased heart [39].

In atrial fibrillation (AF)-induced myocardial fibrosis, overexpression of miR-30a in cardiac fibroblasts induces a significant decrease in expression of snail 1 and periostin. In vivo, expression of miR-30a significantly decreases while the snail 1 and periostin expression level significantly increase in myocardial tissues as myocardial fibrosis degree increases, suggesting miR-30a and snail 1 as potential therapeutic targets for AF-induced myocardial fibrosis [40].

Under stimulation of angiotensin II, miR-503 was up-regulated in cultured cardiac fibroblasts (CFs). Overexpression of miR-503 increased collagen production in CFs, which was the consequence of miR-503-induced expression of CTGF and TGF-β. MiR-503 directly targets Apelin-13, which could inhibit activation of CTGF and TGF-β and collagen production. All of the results together suggest miR-503 facilitates cardiac fibrosis through Apelin-13-TGF-β-CTGF-collagen production pathway [41].

The expression of miR-214 is up-regulated in fibrotic heart tissue and fibroblasts. miR-214 mediates collagen synthesis via mitofusin2 (Mfn2), which is a crucial regulator of tissue fibrosis. Through activation of ERK1/2 MAPK signaling, miR-214 regulates cardiac fibroblasts proliferation [42].

In the overloaded heart, miRNA-15 family is up-regulated. MiR-15 inhibits the TGF-β pathway via targeting TGFβRII and other genes including p38, SMAD3, SMAD7, and endoglin, directly or indirectly. Thus, miR-15 family contributes to cardiac hypertrophy and fibrosis [43]. miR-133a could be downregulated by overexpression of a transcription factor, SRF. miR-133a also acts as a repressor of CTGF. MiR-133a overexpression can block the expression of CTGF. SRF/CTGF/miR-133a axis plays a certain role in the development of cardiac fibrosis, and miR-133a and miR-29b can modulate Col1A1 expression [44,45].

Collagen I and CTGF expression can be regulated by miR-26a. In cardiac fibroblasts, NF-κB activity can be inhibited by inhibition of miR-26a overexpression, while NF-κB repairs miR-26a expression and leads to CTGF and collagen I gene expression reduction, suggesting a feedback regulatory mechanism. The role of miR-26a indicates a potential therapeutic intervention for cardiac fibrosis [46].

Cardiac infarction and angiotensin II both can reduce the expression of miR-101a and miR-101b (miR-101a/b). Expression of miR-101a/b suppresses the collagen production in cardiac fibroblasts. c-Fos and its downstream protein TGF-β1 are found to be targets of miR-101a. miR-101a can mitigate interstitial fibrosis, indicating its therapeutic potential for cardiac disease associated with fibrosis [47,48].

Overexpressing miR-208b improves myocardial functions and inhibits type I collagen and alias aSMA. Research data demonstrated Gata4 as a direct target of miR-208b. By regulating GATA4, miR-208b exhibit protective function against post-infarction myocardial fibrosis [49].

Through direct suppression of high mobility group box 1 (HMGB1), miR-142-3p acts against oxidative pressure induced apoptosis and fibrosis cardiomyocytes. This effect is achieved partly at least via TGF-β1/Smad3 signaling pathway [50].

Circular RNAs (circRNAs) are a type of endogenous noncoding RNA. In diabetic mice myocardium and cardiac fibroblasts (CFs), in response to Ang II, circRNA_010567 was markedly up-regulated. circRNA_010567, sponge miR-141 and miR-141 could directly target TGF-β1, and thus play important regulatory roles in myocardial fibrosis [51].
7 Other biomarkers

Persistently high cardiac troponin is closely associated with cardiac infarction and predicts poor prognosis in various cardiomyopathies. In patients with HCM, patients with higher high-sensitivity cardiac troponin T (hs-cTnT) levels had greater maximum wall thickness and had higher possibility of myocardial fibrosis. Increased hs-cTnT levels are related with fibrosis severity in HCM patients. In 53 patients with HCM, serum levels of hs-cTnT was identified as a direct biomarker of myocardial fibrosis. In 74 patients with nonischemic failing myocardium, ΔcTnT (difference between aortic root and coronary sinus) levels, as well as transcardiac cTnT release, were distinctly higher in patients with detected myocardial fibrosis. In a study conducted in patients with hypertrophic obstructive cardiomyopathy (HOCM), patients with detected fibrosis had significantly higher levels of cardiac troponin I (cTnl) and N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP), with a certain extent of quantitative relationship [52-55].

Osteopontin (OPN) is a multifunctional cytokine that is involved in cardiac fibrosis. It takes part in different physiological processes including wound healing and inflammation. In cardiac biopsies of patients with fibrosis, OPN and miR-21 were significantly increased, accompanied by increased cardiac collagen content, ERK-MAP kinase and AKT signaling pathway activation, myofibroblast activation, and reduced Phosphatase and Tensin Homologue (PTEN) and SMAD7 expression. Overexpression of OPN enhances cardiac fibrosis in vivo and increases expression of collagen-I and α-smooth muscle actin (SMA) in vitro. This process can be mediated by focal adhesion kinase (FAK). In vivo OPN induces cardiac fibrosis in dilated cardiomyopathy (DCM) mice. OPN N-terminal fragment (N-OPN) and OPN C-terminal fragment (C-OPN) are both OPN fragments cleaved by thrombin. N-OPN distinctly promoted fibroblast migration. N-OPN also enhanced expression of SMA, smad signal activity and COL III production. The COL III/COL I ratio and COL III distribution were associated with N-OPN. In patients with hypertensive heart disease (HHD) and HF, OPN was highly expressed whereas very rare in control hearts. OPN was directly correlated with LOX and insoluble collagen. The OPN-LOX axis might contribute to the formation of insoluble collagen. OPN also acts downstream of ROS; elevated ROS level leads to increase of OPN levels [56-60].

ST2 belongs to the interleukin-1 receptor family. The ligand of transmembrane receptor ST2L, interleukin-33, is involved in remission of fibrosis and hypertrophy. In patients with stable CAD, increased ST2 was associated with impaired LV diastolic function [26].

There is a study showing that CTGF could affect the process of hypertension-induced myocardial fibrosis by regulating TGF-β mRNA expression in cardiac tissues [15].

In HCM polymorphisms in nonsarcomeric gene, RETN (−420C>G), and circulating resistin concentration were associated with a higher degree of cardiac fibrosis. Investigating the RETN polymorphism in HCM might help to state the severity of disease [61].

Periostin, a matricellular protein, plays an important role in cardiac remodeling. Periostin mRNA and protein were detected in tissues collected from heart transplant recipients and unmatched donors. Compared with control level, periostin mRNA was increased significantly. Periostin protein expression, distribution and extent were positively associated with myocardial fibrosis. These findings made periostin a potential biomarker of cardiac fibrosis in patients with HF [62].

In a study of 40 patients with HCM, the plasma level of midregional pro-atrial natriuretic peptide (MR-proANP) was positively associated with myocardial fibrosis. The specificity, positive and negative predictive value were satisfying. The results imply that MR-proANP is a potential biomarker of myocardial fibrosis in patients with HCM [63].

8 Conclusion

Myocardial fibrosis contributes to a large number of cardiovascular disorders and at the same time is a pathological feature of many diseases. Detection and diagnosis of myocardial fibrosis are of great clinical value. Lots of molecules alone or in concert play roles in the process of cardiac fibrosis and are used as biomarkers of myocardial fibrosis. As the major feature of MF is dysregulated collagen synthesis, elevated collagens are important markers. Key mediators of collagen metabolism including TGF-β, TIMP, galectin-3, osteopontin, and ST2 are clinically used as indicators of MF as well. MicroRNAs can directly or via other effectors regulate collagen synthesis, as well as molecules involved in development of myocardial fibrosis apart from collagens. Clinical trials have been performed for the last few years to examine the feasibility of these biomarkers in a diagnostic or prognostic way. These biomarkers are not only useful tools in the discovery of MF, they are also often good targets when researchers are looking for effective treatment for cardiac fibrosis.
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