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STATE OF THE ART REVIEW

Cardiac fibrosis in patients with atrial fibrillation: Mechanisms and clinical implications

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Abstract

Atrial fibrillation (AF) is associated with structural, electrical and contractile remodeling of the atria. Development and progression of atrial fibrosis is the hallmark of structural remodeling in AF and is considered to be substrate for AF perpetuation. In contrast, experimental and clinical data on impact of ventricular fibrotic processes in pathogenesis of AF and its complications are controversial. Ventricular fibrosis appears to contribute to abnormalities in cardiac relaxation and contractility, and development of heart failure, a common finding in AF. Given the frequent coexistence of AF and heart failure and the fact that both conditions affect patient prognosis better understanding of mutual impact of fibrosis in AF and heart failure is of particular interest. In this review article, we provide an overview on the general mechanisms of cardiac fibrosis in AF, differences between fibrotic processes in atria and ventricles, and the clinical and prognostic significance of cardiac fibrosis in AF.

Key words: atrial fibrillation, heart failure, cardiac fibrosis

List of abbreviations

AF, atrial fibrillation

CTGF, connective tissue growth factor

DE-CMR, delayed enhancement cardiac magnetic resonance imaging

ECM, extracellular matrix

ET-1, endothelin-1

HF, heart failure

miR, microribonucleic acid

MMP, matrix metalloproteinase

PDGF, platelet-derived growth factor

TGF-β1, transforming growth factor β1

TIMP, tissue inhibitor of matrix metalloproteinase

Introduction

Mechanisms of AF are complex and associated with structural and electrical remodeling in the atria and ventricular myocardium. The key electrophysiological mechanisms of AF include (i) focal firing due to triggered activity (early and delayed after-depolarisations); (ii) multiple re-entries due to shortening of action potential and (iii) heterogeneity of impulse conduction caused by atrial fibrosis. Development and progression of atrial fibrosis is the hallmark of structural remodeling in AF and is considered to be the substrate for AF perpetuation. Advanced atrial fibrosis is associated with more frequent paroxysms of AF, transformation of arrhythmia into a permanent type and reduced effectiveness of antiarrhythmic drug therapy (1,2).

Despite a large body of experimental and clinical evidence supporting role of atrial fibrosis in AF, data on the fibrotic processes in ventricles in patients with AF are limited. The available data indicate that ventricular fibrosis may be at least partly responsible for impaired cardiac relaxation and contractility seen in many AF patients. Cardiac fibrosis may be implicated in complex interactions between AF and heart failure (HF), both of which can be cause(s) and consequence(s) of each other. Given the high frequency of coexistence of both AF and heart failure, and their clear prognostic significance (e.g., increased risk of hospitalization or death related to heart failure deterioration) a better understanding of role of cardiac fibrosis in pathogenesis of AF and its complications is important (3). This review focuses on general mechanisms of the cardiac fibrosis in AF, differences between fibrotic processes in atria and ventricles and on clinical and prognostic impact of cardiac fibrosis in AF.

Mechanisms of cardiac fibrosis

Progressive accumulation of fibrotic tissue in myocardium is one of the major components of cardiac remodeling. Formation and re-distribution of connective tissue fibers modulates myocardial geometry in order to adapt to new conditions of (patho)physiological functioning and to prevent or minimize effects of new mechanical, chemical and electrical stimuli. This adaptation process involves both cellular components of myocardium and extracellular matrix (ECM), an acellular component of the heart, containing a variety of fibers with predominance of collagen (4).

However, excessive ECM production in adults is commonly associated with pathogenesis of cardiovascular diseases, resulting in abnormalities of cardiac contraction and relaxation thus inevitably leading to HF (5). Whilst in the healthy heart collagen deposition is restricted to maintenance of heart architecture, in the process of progression of various cardiac disorders the collagen network undergoes quantitative and qualitative changes leading to excessive accumulation of collagen either in the regions of cardiomyocyte loss (e.g., in myocardial infarction, reparative fibrosis) or diffusely in the myocardium not involved in the focal injury (e.g., in dilated cardiomyopathy, reactive fibrosis) (4,5).

Cardiac fibroblasts and myofibroblasts

Both cellular and extracellular components take part in the remodeling process. Cardiac fibroblasts play a pivotal role in formation of the ECM. They are numerous within the myocardium and can account up to 60% of cells in the cardiac muscle (6). Thus cardiac

fibroblasts even outnumber cardiomyocytes although the latter cells largely determine total myocardial mass.

The population of cardiac fibroblast in healthy adult hearts is maintained at a relatively low level being predominantly represented by resident fibroblasts and epithelial cells subjected to epithelial-to-mesenchymal transition. In pathological conditions, numbers of fibroblasts dramatically increase via differentiation from several cell lineages, including monocytes, endothelial cells, bone marrow circulating progenitor cells and pericytes (7-9).

The physiologic functions of fibroblasts extend beyond metabolism of the ECM. Tight connections between the fibroblasts, fibers of the ECM and other cellular components form multidimensional network acting as an integral sensor of dynamic changes in the various mechanical, chemical and electrical stimuli in the myocardium. In response to these stimuli, this complex system adjusts extracellular matrix turnover, regulates cardiomyocyte hypertrophy and to a smaller extent cardiomyocyte proliferation, but it also triggers activation of fibrotic and inflammatory pathways. Of note, cardiac fibroblasts are able to exhibit various phenotypes depending on the surrounding microenvironment (10).

Cardiac fibroblasts also contribute to electrical remodeling in AF due to their different electrophysiological properties compared to surrounding cardiomyocytes. Fibroblasts are essentially nonexcitable cells but they can transfer currents between cardiomyocytes via connexins. This may result in heterogeneity of current conduction, shortening of action potentials, depolarization of resting cardiomyocytes, and induction of spontaneous phase 4 depolarization (11). Consequently, fibroblasts may be directly involved in re-entry occurrence and perpetuation. Interestingly, computer modeling myofibroblast proliferation in

AF and their electrical interaction with cardiomyocytes were found to be sufficient for re-entry formation even in absence of fibrosis (12).

Myofibroblasts is a group of cells that play a particular significant role in cardiac fibrosis. Myofibroblasts derive from cardiac fibroblasts but they have approximately two-fold higher capacity to synthesize collagen. In comparison to cardiac fibroblasts, myofibroblasts do not appear in healthy myocardium, they are more responsive to proinflammatory and profibrotic stimuli, and are capable of synthesis of a large variety of cytokines and chemokines (13). Importantly, myofibroblasts contain α-smooth muscle actin and adhesion complex (fibronexus). The latter binds myofibroblast internal microfilaments to ECM proteins that helps to provide contractile force to the surrounding extracellular matrix .

ECM turnover and cardiac fibroblast activity are regulated by a range of growth factors, cytokines, and hormones as well as by mechanical stretch and hypoxia. These factors determine fibroblast gene expression, their differentiation and intensity of collagen synthesis.

TGF-β_1 signaling

Amongst the numerous regulatory factors, angiotensin II and transforming growth factor (TGF)-β1 (Figure 1) are the most potent stimulators of collagen synthesis by cardiac fibroblasts (14,15). TGF-β1 produces its effects via binding to TGF-β1 dimerized receptor in the extracellular space, which consists of two receptors – TβRI and TβRII. Ligand-receptor binding results in the cascade of reactions of phosphorylation during which inactive Smad proteins 2, 3 and 4 form Smad complex (16).

The Smad complex then translocates to the nuclei of the target cells where it regulates expression of genes involved in fibrogenesis via appropriate regulatory regions, for example, CTGF and periostin.(17,18) This results into production of so-called matricellular protein, a pro-fibrotic protein secreted into the extracellular matrix. The matricellular protein modulates intercellular and cell-to-matrix interactions that further stimulate extracellular matrix protein synthesis but are not directly involved in ECM structure and mechanical organization or differentiation of cardiac fibroblasts into myofibroblasts (19).

TGF-β-activated kinase 1 (TAK1) is an alternative to Smad pathway for TGF-β1-induced fibrosis. TAK1 is a member of the mitogen-activated protein kinase (MAPK) family (20). Importantly, apart from activation of fibroblast and collagen synthesis TGF-β1 can also induce apoptosis of cardiomyocytes (21).

Of note, angiotensin II is not able to induce cardiac hypertrophy and fibrosis in the absence of TGF-β1, but it up-regulates TGF-β1 synthesis, Smad2 phosphorylation, nuclear translocation of the Smad complex and increases Smad DNA-binding activity. TGF-β1 in turn can directly stimulate expression of angiotensin II type 1 receptor (22). Angiotensin II also predisposes to fibrosis by promoting expression of pro-fibrotic factors, such as endothelin-1. In conjunction with aldosterone angiotensin II promotes oxidative stress (i.e. excess production of reactive oxygen species) and inflammation, mainly by activation of NADPH oxidase (23,24).

Matrix metalloproteases and tissue inhibitors of matrix metalloproteases

Remodeling and maintenance of the extracellular space includes not only synthesis but also coordinated degradation of the extracellular matrix proteins. Matrix metalloproteases (MMP)

and their tissue inhibitors, synthesized by cardiomyocytes and cardiac fibroblasts are intimately involved in maintenance of the extracellular matrix homeostasis (25). Indeed, MMP expression increases in a time-dependent manner with left ventricular dysfunction and dilatation (26). Overexpression of MMP-1 has been observed to cause compensatory hypertrophy and increased collagen concentration within the myocardium. In contrast, targeted deletion of MMP-2 results in amelioration of left ventricular remodeling (27). Not surprisingly MMP activity increases in line with TGF-β1 expression within the myocardium and correlates with the level of inflammation and oxidative stress (28).

Furthermore, collagen and matrix fragments produced by the action of MMP-1 themselves form bioactive molecules, so called matrikines, and release ECM-embedded pro-inflammatory and pro-fibrotic factors. They promote fibroblast activation and transition to a myofibroblast phenotype and effectively stimulate connective tissue synthesis by serving as ligands of leucocyte integrins and other cell activating receptors (25,29). The latter explains progression of fibrosis when high MMPs activity despite the primary MMP function directed towards matrix degradation. MMP activity is regulated via TIMPs and reversion-inducing-cysteine-rich protein with Kazal motifs (RECK). RECK overexpression was found to blunt angiotensin II-induced MMP activation and cardiac fibroblast migration (30).

Regulatory role of microribonucleic acids

Nuclear miRs play important regulatory roles in cardiac remodeling (31). They are referred to as endogenous, single stranded, short (approximately 22 nucleotides), noncoding RNAs. MiRs degrade or inhibit at the post-transcriptional level the translation of their target messenger RNAs, thus regulating gene expression (32).

Several miRs are involved in the fibrogenesis. miR-133 and miR-30 regulate cardiac fibrosis by repressing CTGF expression. They were found to be down-regulated in left ventricular hypertrophy that was associated with increased CTGF expression (33). miR-133 knockout mice developed advanced fibrosis and heart failure with predisposition to sudden death (34). On the contrary overexpression of miR-133 results in decreased collagen synthesis by fibroblasts, reduced myocardial fibrosis and apoptosis (33,35). miR-21 is involved in up-regulation of one of the pro-fibrotic pathways (ERK) and promotes MMP-2 expression (36,37). Interestingly, it also produces protective effects, including defense against oxidative stress, inhibition of pro-apoptotic factors and increased expression of anti-apoptotic genes (38). Finally, miR-29 is associated with the collagen type I and III deposition. Upregulation of miR-29 leads to downregulation of these proteins and vice versa (39).

Inflammation

AF is common in patients with overt inflammatory states of cardiac and noncardiac location (e.g., myocarditis, pericarditis, pneumonia, inflammatory bowel disease), but low grade subclinical inflammation (e.g., in coronary heart disease) also contributes to pathogenesis of the arrhythmia (Figure 2) (40). Whether AF is a cause or consequence of the inflammatory process, the latter is related to oxidative stress perpetuated by myocardial infiltration with inflammatory cells (e.g., macrophages) and release of reactive oxygen species by cells of the ECM. Inflammation is further exacerbated by activation of renin-angiotensin-aldosterone system followed by activation of NADPH oxidase. These processes consequently trigger TGF-β1 signaling, structural and electrical remodeling (41). Various inflammatory cytokines and chemokines, such as interleukins 1 and 6, tumor necrosis factor α, monocyte

chemoattractant protein 1, are upregulated in AF and linked to progression from paroxysmal to chronic AF and AF recurrence post-cardioversion (40).

Inflammation plays a particular role in postoperative AF (e.g., after CABG, valvular replacement surgery) and post catheter ablation. In a recent meta-analysis of 925 postoperative patients serum C-reactive protein was a potent predictor of new-onset AF (42). Similarly, a meta-analysis of 7 studies of post-ablation patients confirmed predictive role of C-reactive protein for AF recurrence (43).

Aging and cardiac fibrosis

Prevalence of AF significantly increases in the elderly. Cardiac aging is a complex process featured by progressive decline in heart functions and ventricular and atrial remodeling. This process includes reduction in cardiomyocyte numbers, hypertrophy of the remaining cardiomyocytes, alteration of myofibrillar orientation, proliferation of cardiac fibroblasts, and collagen deposition. Progressive fibrosis is a hallmark of aging heart as confirmed by increased collagen volume fraction in myocardium and imaging in animal and human studies (44).

Age-related cardiac fibrosis reflects multiple processes that accompany cardiac senescence, chronic activation of the renin-angiotensin-aldosterone axis, excessive β-adrenergic and endothelin signaling, activation of TGF-β1 pathway, disruption in intracellular calcium homeostasis, cardiomyocyte apoptosis, recruitment of mononuclear cells and fibroblast progenitors, downregulation of mitochondrial NAD-dependent deacetylase sirtuin-1 (45).

Increased generation of reactive oxygen species and diminished antioxidant capacity are major contributors to age-related myocardial remodeling (Figure 3). Oxidative molecules derive from oxidative phosphorylation processes in mitochondria, increased NADPH oxidase activity, uncoupled nitric oxide synthase function, lipid oxidation within peroxisomes, and upregulation of cyclooxygenases and xanthine oxidase (46). Chronic oxidative stress leads to persistence of low-grade inflammation thus further accelerating cardiac fibrosis. Among important regulators of aging-related processes is miR-34a with PNUTS (also known as PPP1R10) being the target. Ageing-induced expression of miR-34a and inhibition of PNUTS is associated with telomere shortening, DNA damage, cardiomyocyte apoptosis, and impaired functional recovery after ischemic injury (47). Hence, profibrotic mechanisms involved in AF pathogenesis are clearly enhanced by aging.

Cardiomyocytes – cardiac fibroblasts communication

Close interaction between cardiomyocytes and cardiac (myo)fibroblasts is essential for their function. These interactions are facilitated by multiple paracrine signals including those predisposing to fibrosis (Figure 4). Cardiomyocytes, cardiac fibroblasts, and myofibroblasts share many common molecular pathways (e.g., mediated by angiotensin II, TGF-β1, endothelin, cytokines). However response to signaling may vary depending on cell type: hypertrophy and reduced cell survival of cardiomyocytes are promoted by angiotensin II–induced release of TGF-β1 and endothelin-1 from fibroblasts while angiotensin II was also found to trigger release TGF-β1 and endothelin-1 from cardiomyocytes and to stimulate fibroblasts proliferation, their differentiation to myofibroblast phenotype and synthesis of components of ECM.(48) Greater proliferation of fibroblasts was observed around

cardiomyocytes expressing AT1 receptors compared to cells with knocked-out AT1 gene (49).

There are also differences between the cells in receptor density and receptor kinase activity, which may interfere with final effect of effector molecules. For example, fibroblasts are known to carry more receptors to angiotensin II then cardiomyocytes (49). Multiple other regulatory substances are implicated in fibroblast-cardiomyocyte interplay (e.g., fibroblast growth factor 2, interleukins, natriuretic peptides, and miR) (48). Some stimuli are attributed predominantly to fibroblasts (e.g. PDGF, FGF-2, activation by mechanical stretching) while abnormalities in calcium handling are largely seen in cardiomyocytes (50).

Cardiac remodeling requires fibrosis as an essential part, and is a complex process that also incorporates multiple other pathways, such as hypoxia signaling, osteoprotegerin/RANK/RANKL axis and Ca signaling among others. Detailed description of these pathways is beyond the scope of the current review.

In summary, ECM represents macromolecular metabolically active dynamic network of fibers (predominantly collagen) and cells (predominantly cardiac fibroblasts with a capacity to differentiate into myofibroblasts) that is essential for normal heart functioning. Cellular component of the extracellular matrix is linked to the fibrillar one and, hence, is capable to respond to mechanical stretch and stress as well as to a variety of autocrine and paracrine stimuli by change in their proliferation, migration and intensity of collagen synthesis. These processes may have unfavorable role in cardiac remodeling and the pathogenesis of cardiovascular diseases.

Atrial fibrosis in atrial fibrillation

A variety of signaling systems are involved in promotion of atrial fibrosis as evidenced by numerous human and animal data (Tables 1-2). Atrial fibrosis may develop as part of AF-related structural remodeling as well as consequence of other cardiovascular diseases, which result in atrial overload and stretch. Conditions associated with atrial fibrosis include hypertension, valvular heart disease and HF, and they cause broadly similar histologic changes in atrial myocardium (51). However, the precise causality between AF and atrial fibrosis may be difficult to establish.

Experimental data also yielded controversial results. For example, some models of atrial tachyarrhythmia demonstrated marked biatrial dilation with changes in atrial architecture and myocyte characteristics, such as loss of myofibrils, accumulation of glycogen, changes in mitochondrial number, shape and size, fragmentation of sarcoplasmic reticulum, dispersion of nuclear chromatin whilst the interstitial space remained unaltered without evidence of increased connective tissue content (52). In contrast, more recent studies of rapid atrial pacing demonstrated upregulation of potent profibrotic factors as angiotensin II and TGF-β1 (53) and increased collagen content in the atrial interstitium (54). The discrepancy might be attributable to the time required for development of detectable fibrosis after initiation of profibrotic pathways. For example, in a mice model of heart failure at 8 weeks there were no signs of histological fibrosis in the left atrium despite increased expression of genes related to fibrosis (55). This discrepancy can also suggest existence of still poorly understood mechanisms of inhibition of fibrosis despite activation of profibrotic genes.

Background cardiovascular disease causing HF can be associated with more advanced atrial changes. For example a model produced by combination of rapid atrial pacing with mitral regurgitation, inevitably resulted in intercellular space expansion in the left atrium and AF (56). This observation is consistent with a HF model of AF caused by ventricular tachypacing where connective tissue contained increased numbers of fibroblasts, more collagen, and showed signs of degeneration and necrosis in comparison to atrial pacing model (57). In a study by Cardin et al ventricular tachypacing led to approximately 10-fold overexpression of collagen mRNA in atrial cardiomyocytes, in comparison to atrial pacing noted as early as at 24 hours and progressing further at 2 weeks. Of note, 8 collagen genes were upregulated more than 10-fold, fibrillin 8-fold and MMP2 4.5-fold at 2 weeks but there were no changes in their expression at 24 hours (58). Also, TGF-β1 levels in failing hearts in animals appeared to be higher than in the non-failing heart (59). Although development of atrial fibrosis has been well documented in several animal models of AF associated with HF the cardiac fibrosis is unlikely to be the sole mechanism of HF, including HFpEF. For instance, predominant electrical remodeling with minimal if any evidence of atrial fibrosis and preservation of ventricular contractility was seen in a sheep model of prolonged persistent AF induced by intermittent atrial tachypacing, but no significant tachycardia during the observation period (60).

Thus, atrial remodeling is the mainstay for initiation and perpetuation of AF. Atrial structural and functional changes may develop as a result of underlying cardiac conditions, pathological systemic processes, or AF itself. Atrial remodeling also commonly occurs as part of age-related processes. However, relationship between AF course and atrial fibrosis is complex and nonlinear, meaning that higher collagen deposition within the atria does not always cause more frequent paroxysms of the arrhythmia and its progression towards persistent or

permanent type. Plethora of mechanisms (hemodynamic alterations, mechanical stretching, changes in hormones, growth factors, proinflammatory cytokines, etc.) modulates severity of atrial fibrosis. A large body of experimental and clinical data has already provided insights into key mechanisms of atrial fibrosis.

Extracardiac and the genetic factors contributing to atrial fibrosis

Multiple 'non-cardiac' factors predispose to fibrosis in AF, including obesity, metabolic syndrome, use of toxic substances, athlete heart, obstructive sleep apnea, systemic inflammation, and thyrotoxicosis. Ultimately all these factors affect the myocardium (61). Recently diabetes, a disease associated with specific cardiomyopathy and excessive cardiac fibrosis was shown to triple risk of AF in obese individuals (62). Obesity leads to electrical and structural atrial remodeling and is associated with diastolic ventricular impairment, atral dilatation and myocardial lipidosis (63). Obesity in AF is related to delay and significant heterogeneity in atrial conduction, atrial inflammatory infiltration and interstitial fibrosis. Pathways underlying these changes include activation TGF-β1 signaling, oxidative stress, upregulation of PDGF and endothelin (64). Pericardial fat envelope could produce constriction effect thus disturbing cardiac relaxation. Some adipokines have clearly profibrotic properties (e.g. activin A, a member of TGF-β1 superfamily), whilst AF itself affects adipocyte-related gene expression facilitating expansion of cardiac fat (65).

Obstructive and central sleep apnea are also associated with atrial remodeling and AF (66). Animal models obstructive sleep apnea showed substantial connexin-43 downregulation, altered expression of channel proteins with net effects of shortening of atrial refractory period, slowing of atrial conduction, cardiomyocyte apoptosis and cardiac fibrosis. Repeated

apnea episodes associated with chronic hypoxia trigger production of strongly profibrotic hypoxia-inducible factors 1α and 2α (67). Moreover, such patients have increased angiotensin-converting enzyme and IL-6 expression with inhibited degradation of atrial collagen via MMP-2 (68). Also sleep apnea leads to myocardial hypertrophy and diastolic dysfunction, thus further potentiating development of HF in presence of AF (69,70). There are multiple other factors contributing to atrial remodeling and chronic low-grade inflammation with increased propensity to AF (e.g. chronic kidney disease, diabetes) (11).

AF has a genetic predisposition. The genetic background of AF is complex with multiple pathways involved. In 'lone' AF, i.e. in the absence of apparent cardiovascular disease, histological and imaging evidence suggests similar extent of atrial fibrosis as in AF patients with structural heart disease.(71) A specific fibrotic atrial cardiomyopathy as an underlying condition predisposing to AF development and persistence has been suggested and it is likely to have genetic background.(72,73). One of genes involved in the atrial development is the PITX2 gene. PITX2 deficiency results in formation of enlarged atrial with thin walls and prominent deficiency in expression of ion channels (74). AF is also related to polymorphisms in genes involved in fibrotic pathways, such as genes responsible for modulation of synthesis of interleukins 1 and 6 (2). Nonetheless robust evidence on target genes directly responsible for AF related cardiac fibrosis is lacking at present with further research warranted in this direction.

Ventricular fibrosis in atrial fibrillation

Association of AF with ventricular fibrosis is less established than for atrial fibrosis. Ventricular fibrotic changes are more pronounced in AF patients than in subjects with sinus

rhythm, both with magnetic resonance or ultrasound imaging (75,76). Also more extensive changes were found in patients with permanent or persistent arrhythmia compared to paroxysmal AF (75-77). These reports were confirmed by animal data showing implication of ventricular fibrosis in cardiac remodeling and rate control in AF (78). Avitall et al observed more extensive ventricular fibrosis in AF when ventricles were not protected from high atrial rate with the atrioventricular node ablation than in the ablated animals (79).

Atrial and ventricular fibrosis in AF are likely to share many common mechanisms, although extent of the changes may vary between the two parts of the heart. In transgenic mice with TGF-β1 overexpression TGF-β1 upregulation was more pronounced in atria than in ventricles. High TGF-β1 levels were associated with enhanced expression of only two profibrotic genes in ventricles in contrast to 80 genes in the atria. Interestingly, TβRI, TβRII and Smad protein levels were similar in ventricles and atria, but Smad2 phosphorylation was increased in atria only, making them prone to development of selective fibrosis (59). Several have been suggested as mechanisms for different effects of TGF-β1 in ventricles versus atria (Table 3) (59). Importantly, the processes above were reported in intact ventricles. In case of combination of TGF-β1 overexpression with other pathophysiological stimuli (such as those seen in HF), TGF-β1 mediated profibrotic ventricular effects appear to be enhanced, although atrial fibrosis still predominated (59). Also, atrial fibroblasts have been found to be more susceptible to PDGF, angiotensin II and endothelin 1 indicating that their activity was premodulated by local atrial factors (80). There is a differential impact of hemodynamic changes (e.g., stretch) and the precise biochemical processes on fibroblast activity in ventricles and atria remain unclear at present.

In summary, the cardiac profibrotic microenvironment in AF is unlikely to be strictly isolated by the atria, and ventricular myocardium is likely to be affected as well. Even within atrial myocardium fibrotic changes take years to become detectable with currently available diagnostic methods. With considerably higher myocardial thickness of the ventricles compared to atria detectable ventricular fibrosis may require prolonged time to develop unless it is amplified by co-existing pathological conditions such as hypertension, coronary artery disease and heart failure.

Imaging of cardiac fibrosis

Significant technological advances allow more possibilities for characterization and quantification of focal and diffuse cardiac fibrosis, which was only possible with biopsy in the past. The most commonly used late (delayed) gadolinium enhancement cardiac magnetic resonance (DE-CMR) imaging is based on difference in properties of healthy myocardium and areas of fibrotic tissue to clear gadolinium, that is T1 relaxation time shortening agent. Fibrotic tissue is characterized by slowing of gadolinium washing-out resulting in greater signal compared to surrounding 'reference' tissue. The latter, however, poses a problem of finding appropriate 'reference' tissue for quantification of diffuse cardiac fibrosis (81,82).

Another CMR-based method, T1 mapping was developed to overcome the problem. T1 mapping is a calculation of a post-contrast myocardial T1 time by imaging a given plane with sequentially increasing inversion times without the need to compare the results to a normal reference tissue before or after the use of a contrast agent. This allows demonstration of diffuse fibrotic fibers with might appear nearly isointense using delayed enhancement.(81,82)

Despite being the gold standard CMR is not widely available. Hence echocardiography remains an alternative for evaluation of cardiac fibrosis based on dependency of acoustic properties on myocardial composition. Collagen causes ultrasound scattering and attenuation, which can be measured as integrated backscatter (83). Furthermore, with the echocardiography functional assessment as strain peak and strain velocity - parameters which characterize reservoir performance and were found to predict the degree of fibrosis detected in histological specimens and via CMR (84,85).

Clinical implications and prognostic impacts of atrial fibrosis

Atrial remodeling including excessive fibrosis has major clinical implication in AF. Numerous studies link more extensive atrial interstitial fibrosis to lower effectiveness of AF catheter ablation and MAZE procedure, increased risk of development of postoperative AF and impaired postprocedural recovery of atrial function (Table 4). Interestingly, delayed enhancement of atrial myocardium is helpful for evaluation of post-ablation atrial fibrosis and its relation to left atrial reverse remodeling on sinus rhythm (86). Patients undergoing pulmonary vein isolation had higher AF recurrence rate with a lesser degree of left atrial and pulmonary vein scarring on DE-CMR (87).

There are limited data on association of atrial fibrosis with stroke risk in AF patients. An association was found between percentage of atrial fibrosis assessed via DE-CMR and higher $CHADS_2$ score and stroke history (88). Moreover, in their population (387 patients, 36 strokes) adding left atrial fibrosis to the risk model (i.e. $CHADS_2$, but not accounting for previous stroke due to retrospective nature of the study) improved the c-statistic form 0.58 to 0.72. This was consistent with another study which demonstrated better prediction of left

atrial thrombosis or spontaneous echocardiographic contrast by addition of the degree of atrial fibrosis to either the $CHADS_2$ or CHA_2DS_2-VASc scores (89). However routine use of expensive and not universally available DE-CMR for stroke prediction is not practical at present in comparison with available and recommended stroke risk assessment tools, and the approach clearly needs further validation.

Galectin–3 involved in regulation of fibrosis was found to be associated with the LA volume index and AF (OR 87.5, 95% CI 6.1-1265) and, also, to be significantly higher in patients with persistent AF then in those with paroxysmal type of arrhythmia (90).

Clinical implications and prognostic impact of ventricular fibrosis

Ventricular fibrosis has detrimental impact on both systolic function (due to replacement of apoptotic and necrotized myocardium) and diastolic function (due to increasing stiffness and decreasing compliance) (14). This makes the ventricular fibrosis relevant both in the context of heart failure with preserved or reduced ejection fraction.

Although scarce data are available on histological assessment of ventricular myocardial fibrosis in patients with AF small case series of ventricular biopsies suggest presence of active myocarditis or nonspecific necrotic/fibrotic changes in patients with ‘lone’ AF (91). It is likely that most of ‘lone’ AF cases have, in fact, background myocardial pathology, which cannot be easily determined using routine tests and its is reflective of primary electrical disturbances and subclinical cardiomyopathies, likely associated with myocardial fibrosis.

Contemporary markers of ventricular fibrosis (e.g., beta-galactoside-binding lectin galectin-3) are studied increasingly in patients with HF and they have shown predictive value for

adverse outcomes but specific relevance in the context of AF remained to be established (92,93).

Prognostic significance of markers of collagen turnover, was assessed in hypertensive heart disease, hypertrophic cardiomyopathy and HF (94,95). However, the major limitation of blood markers of collagen turnover is that they are not cardiac-specific and may not accurately reflect myocardial collagen content. Hence, the results are often conflicting, depending largely on selection of study cohorts (e.g., exclusion of patients with hepatic and kidney dysfunction, pulmonary fibrosis, osteoporosis, metastatic bone disease, etc. or measuring cardiac gradient of collagen markers (i.e., blood from coronary sinus vs. systemic circulation) (96).

It is even more problematic or even impossible to distinguish atrial and ventricular contribution to circulating levels of byproducts of collagen synthesis and degradation. Consequently attribution of elevated markers of collagen turnover to AF-related atrial fibrosis alone might be not entirely correct.

For example, in the I-PRESERVE trial collagen substudy that included 29% of AF patients procollagen type I amino-terminal peptide and procollagen type III amino-terminal peptide were predictive of all-cause mortality and cardiovascular hospitalizations, but this association lost significance after adjustment for other confounders (97).

Similarly, majority of imaging-based studies on evaluation of ventricular fibrosis focused on patients with arterial hypertension, dilated and hypertrophic cardiomyopathy, HF, but only few directly addressed AF patients. Moreover, those few studies with AF included mostly

patients referred for AF ablation, which is currently indicated for patients with paroxysmal or persistent arrhythmia resistant to antiarrhythmic treatment. Thus, these studies may not be representative of the whole AF population.

Recently Neilan et al revealed association between the presence (HR 5.08, 95% CI 3.08-8.36) and extent (HR 1.15, 95% CI 1.10-1.21) of left ventricular late gadolinium enhancement on magnetic resonance imaging and all-cause mortality (n=664, median follow-up 42 months, mean LVEF 56±10%) (98). The observed associations were even stronger when patients with the evidence is ischemic heart disease were excluded from analysis (98). The major limitation of this study was inclusion of patients selected for AF ablation with a median duration of arrhythmia since onset of 50 days.

Another study that involved patients with arterial hypertension and longer AF duration (median of 37 months) and based on CMR T1 mapping found the left ventricular extracellular to be independently predictive of AF recurrence as well as of the composite end point of AF recurrence, admission with HF, and death (HR 1.35, 95% CI 1.21-1.51).(99) Prognostic value of diffuse ventricular fibrosis for AF recurrence after ablation procedure was further confirmed by McLellan et al with a cut off level of postcontrast ventricular T1 time <380 ms being related to better outcome (100). In a small cohort of patients without left ventricular fibrosis as evidenced by the absence of late gadolinium enhancement on CMR in patients with systolic HF significant improvement of left ventricular function was observed after AF catheter ablation. However the study had no comparator group with established ventricular fibrosis to assess impact of sinus rhythm restoration (101).

Thus far, similarly to atrial fibrosis it is unclear whether AF is a trigger of the present profibrotic pathways in the left ventricle or merely a marker of preexisting fibrotic changes, or even both.

Treatment approaches to reduce cardiac fibrosis

Given that angiotensin II is a potent stimulator of profibrotic pathways, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists and mineralcorticoid receptor antagonists deemed to reduce fibrosis progression. This was, in fact, supported by several animal studies, but not by clinical trials, so far. Retrospective analyses and meta-analyses of randomized trials showed inconclusive results both for primary and secondary AF prevention. It has been also accepted that overall primary prevention of AF with these agents is more feasible then than the secondary prevention as myocardial fibrosis is more likely to be slowed down than reversed (102,103).

Therefore inhibitors of angiotensin axis are only recommended for AF management when the arrhythmia is associated with other underlying conditions associated with myocardial fibrotic remodeling such as arterial hypertension with left ventricular hypertrophy, systolic HF and they are not recommended in patients without apparent cardiovascular disease (e.g., ‘lone’ AF) (104).

Many other components of profibrotic cardiac pathways (e.g., TGF-β1, PDGF, etc.) represent attractive therapeutic targets. Their suppression with either antibody blockade or oligonucleotide interference was shown to reduce interstitial fibrosis in animal experiments.

Nonetheless experience from the animal data need to be confirmed by clinical trials and better understanding of details of fibrotic pathways is required.(15)

Conclusion

AF is associated with fibrotic processes both in atria and ventricles. Despite common pro-fibrotic pathways, signaling in ventricular and supraventricular parts of the heart seems to be different. Atrial fibrosis may precede development of AF, which in turn results in further progression of atrial remodeling. Structural heart disease appears to have greater impact on both atrial and ventricular fibrosis than arrhythmia per se but it allows persistent activation of pro-fibrotic stimuli. Whilst the role of atrial fibrosis in AF is well documented, the implication of ventricular fibrosis in pathogenesis and outcome of conditions associated with AF clearly requires further research.

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Table 1. Studies on mechanisms of atrial fibrosis in atrial fibrillation in humans

Reference	Number (AF/SR)	Sample tested	LVEF, % (AF/controls)	Results (AF vs. controls)	Observed associations
Adam et al, 2010 (105)	5/5	LAA	61±6/59±6	↑collagen, CTGF, NADPHox, Rac1, N-cadherin, connexin 43, angiotensin II	NA
Adam et al, 2012 (106)	5/5	LAA	61±6/59±6	↑miR-21	miR-21 with collagen, CTGF, Rac1, LOX, angiotensin II
Cao et al, 2013 (107)	48/24	RAA	63±5 (paroxysmal), 64±5 (persistent) / 64±3	↑OPG, RANKL, RANK, RANKL/OPG ratio	(In AF) OPG, RANKL, RANK, RANKL/OPG ratio with collagen type I, III
Dawson et al, 2013 (108)	17/30* 17/19	Plasma RAA	60±2/69±1	↓miR-29b ↓miR-29b in chronic AF	NA
Gramley et al, 2007 (109)	42/104	RAA	48±12	↑collagen content, activity ↔MMP2, MMP9 (mRNA and protein levels), ↓PAI, TIMP1 and 2 (mRNA) with ↑ duration of AF	NA

Gramley et al, 2010 (67)	42/116	RAA	50±11/47±12	↑collagen content, HIF-1α, HIF-2α, VEGF, KDR, pKDR and microvessel density	NA
Gramley et al, 2010 (110)	61/102	RAA	48±11	↑collagen content, early ↑ and later ↓ responsiveness to TGF-β1 with ↑ duration of AF: initially ↑TGF-β1 (mRNA and protein), TβRII, phSmad2, Smad4 (protein) followed by a ↓TβRI phSmad2 (protein) and ↑Smad7 (protein)	NA
Kallergis et al, 2008 (111)	70/20	Serum	60±4 (paroxysmal), 56±9 (persistent)/60±5	↑CITP, CICP, TIMP1	NA
Ko et al, 2011 (112)	10/10	RAA	53±15/44±17	↑collagen content, CTGF (protein and mRNA)	NA
Li et al, 2013 (113)	28/12	RA	NA	↑collagen content, TGF-β1, Smad3 and CTGF	TGF-β1, CTGF (mRNA and protein) with collagen content, TGF-β1, CTGF
Mayyas et al, 2010 (114)	32/21	LAA	51±2/53±3	↑ET-1 ↔ET_AR or ET_BR	ET-1 with LA size, AF persistence
Nishi et al,	16/13	RA	70±8 (unsuccessful	↑miR-21, miR-23b, miR-199b, miR-208b	miR-21with collagen content

2013 (115)			MAZE), 53±15 (successful MAZE)/ 62.0±9		
Okumura et al, 2011 (116)	50/0	Serum	NA	↓hsCRP, IL6, ANP, BNP ↑MMP2, TIMP2, CITP during follow-up	MMP-2 with AF recurrence
Polyakova et al, 2008 (117)	24/24	RA, RAA	46±10/46±13	↑collagen content, MMP2, MMP9, TIMP1, TIMP2, RECK, TGF-β1, Smad2 and phSmad2	NA
Qu et al, 2009 (118)	20/20	RA	51±15/63±14.63	↑collagen content, TNFα, IL6 and NFκB activity	NFκB activity with TNFα, IL6 and collagen content
Rahmutula et al, 2013 (59)	17/NA	RA	NA	↑TGβ1 and TGF-β1 signaling-related genes (phSmad2, Smad6, Ang II, etc.)	NA
Richter et al, 2011 (119)	30/0	Serum	62±2	↑MMP9, TGF-β1, PIIINP after ablation	PIIINP with AF recurrence; MMP9, TGF-β1 with ablation-induced LA volume reduction; MMP9 with RF energy on ablation
Rudolph et al,	34/35	Plasma,	49±89/52±9	↑MPO	NA

2010 (120)		RAA			
Swartz et al, 2012 (121)	18/36	LAA, RAA, serum	49±12/51±8	↑collagen content, collagen type I, III, TGF-β1, Ang II (mRNA) ↑PICP, PIIINP	Collagen content with PICP
Wang et al, 2015 (122)	30/17	LAA	63±7/70±4	↑miR-146b-5p, MMP 9, collagen content; ↓TIMP-4	miR-146b-5p with TIMP-4 and collagen content
Wilhelm et al, 2006 (123)	30/20	RAA	NA	↔collagen content	NA
Wu et al, 2013 (124)	200/0	Plasma	53±10/57±6†	NA	TGF-β1 with AF recurrence
Xi et al, 2013 (125)	83/52†	RAA	62±6/30±5	↔RANK, RANKL	RANK, RANKL, RANKL/OPG ratio with collagen content
Xie et al, 2013 (126)	22/15	LA,	55/60	↑fibrocytes, collagen I and αSMA	Fibrocytes with collagen content, LA volume index
Xu et al, 2009 (127)	27/18	LA, RA	54±9/59±12	↑collagen type I, MMP1 and MMP-9 (mRNA) ↓TIMP1 (mRNA)	Collagen type I (mRNA) with atrial diameter;

					MMP1, MMP9 (mRNA) with TIMP1 (mRNA)

* Recurrent AF / nonrecurrent AF; † chronic AF / AF with subsequent SR recovery.

↑, increased; ↓, reduced; ↔not changed; AF, atrial fibrillation; ANP, atrial natriuretic peptide; α-SMA, α-smooth muscle actin; BNP, brain natriuretic peptide; CICP, collagen type I C-terminal propeptide; C-terminal telopeptide CITP, collagen type I C-terminal telopeptide; COL1A1, gene encoding alpha-1 type I collagen; COL3A1, gene encoding alpha-1 type III collagen; CTGF, connective tissue growth factor; ET-1, endothelin-1; ET_AR, type A ET-1 receptor; ET_BR, type B ET-1 receptor; hsCRP, high sensitive C-reactive protein; HIF-1α, hypoxia-inducible factor 1α; HIF-2α, hypoxia-inducible factor 2α; IL-6, interleukin-6; KDR, VEGF receptor 2; LA, left atrium; LAA, left atrial appendage; LOX, lysyl oxidase; miR, microribonucleic acid; MMP, matrix metalloproteinase; MPO, myeloperoxidase; mRNA, messenger ribonucleic acid; NA, not available; NADPHox, nicotinamide adenine dinucleotide phosphate oxidase; NFκB, nuclear factor kappa B; OPG, osteoprotegerin; PICP, procollagen type I C-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; PAI, plasminogen activation inhibitor; phSmad, phosphorylated Smad; pKDR, phosphorylated KDR; RA, right atrium; RAA, right atrial appendage; Rac1, Ras-related C3 botulinum toxin substrate 1; RANK, receptor activator of NFκB; RANKL, RANK ligand; RECK, reversion inducing cysteine-rich protein with Kazal motifs; Smad, transcriptional factor, named by fusion of C. elegans Sma protein and Drosophila Mad (mothers against decapentaplegic) protein, in reference to its sequence similarity to these proteins; TβRI, type I TGF-β1 receptor; TβRII, type II TGF-β1 receptor; TGF-β1, transforming growth factor beta 1; TIMP, tissue inhibitor of MMP; TNFα, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

Table 2. Studies on mechanisms of atrial fibrosis in atrial fibrillation in animal experiments

Reference	Number (experiment/control)	Samples tested	Model	Results (experiment vs. controls)
Abed et al, 2013 (64)	30	LA, LAA, RA, and RAA	High calorie diet	↑AF inducibiity, LA volume, collagen content, inflammatory infiltrates, lipidosis, ET-1, ET_AR, ET_BR, TGF-β1, PDGF with ↑ adiposity
Adam et al, 2012 (106)	NA	LA	Tx, Rac1 overexpression	↑collagen content, miR-21, spontaneous AF
Cardin et al, 2012 (128)	NA	Myocardium	Coronary artery ligation	↑miR-21, LA dilation and collagen content, AF inducibility
Dawson et al, 2013 (108)	57/37	LA	Ventricular tachypacing; Tx, miR-29b knockout	↓miR-29b, miR-133a, miR-133b; ↑collagen content ↑collagen content, COL1A1 (mRNA)
He et al, 2011 (53)	8/8	LA	Atrial tachypacing	↑Angiotensin II, TGF-β1, phSmad2/3, Arkadia, hydroxyproline expression;

				↓Smad7 expression
Kiryu et al, 2012 (129)	10/5	LA, RA	Atrial tachypacing	↑CTGF, collagen type I, III; ↔TGF-β1
Ko et al, 2011 (112)	6/6	LA, RA	Atrial tachypacing	↑Angiotensin II, CTGF (protein and mRNA)
Li et al, 2012 (54)	21/21	LA	Atrial tachypacing	↑collagen content, chronic inflammation; ↓miR-133 and miR-30
Rahmutula et al, 2013 (59)	15/15	Myocardium	Tx, TGF-β1 overexpression	↑LA fibrosis, AF inducibility, TGF-β1 signaling-related genes in atria (TIMP, MMP, collagen, Smad2 or 3, TβRI and II)
Rudolph et al, 2010 (120)	NA	LA	MPO knockout, angiotensin II pretreatment	↓collagen content, MPO, MMP2, MMP9 and AF susceptibility
Saba et al, 2005 (130)	32/37	Myocardium	Tx, TNFα overexpression	↑collagen content, AF inducibility
Verheule et al, 2004 (131)	30/30	Myocardium	Tx, TGF-β1 overexpression	↑LA fibrosis, AF inducibility

PDGF, platelet derived growth factor; Tx, transgenic; other abbreviations as in Table 1.

Table 3. Suggested mechanisms of predisposition of atrial versus ventricular myocardium to fibrosis

Gene expression
Higher expression of genes encoding extracellular matrix (e.g., fibronectin, laminin, fibulin), cell signaling (PDGF, PDGF receptor, angiopoietin, VEGF), etc. in fibroblasts
Signaling pathways
Greater signaling via canonical TGF-β1 pathway including enhanced receptor binding, receptor-kinase activity, SMAD2/3 phosphorylation, reduced expression of the inhibitory SMAD7 in atrial myocytes with further stimulation of atrial fibroblasts
Higher level of endogenous AT1 receptor and greater enhancement of receptor level following pathological influences
Differential expression of adapter/scaffolding proteins (β-arrestin-1, G-proteins) involved in the AT1 receptor-dependent aldosterone synthesis and secretion
Higher expression of PAI1
Cellular proliferation
Higher myofibroblast density in healthy and diseased hearts with faster cell surface area being increased, distinct morphology at confluence and greater α-SMA and vimentin expression
Higher proportion of fibroblasts in mitotic phases and displaying enhanced gene expression of fibroblast-selective markers
Greater proliferation response of atrial fibroblasts for a range of growth factors (e.g., PDGF, angiotensin II, ET-1, and TGF-β1)

AT1, angiotensin II receptor type 1; PAI-1, plasminogen activator inhibitor 1; other abbreviations as in Tables 1 to 2.

Table 4. Studies on prognostic significance of atrial fibrosis in AF patients

References	Number of patients	Duration of follow-up	Evaluation of atrial fibrosis	Intervention	Study outcome	Association of atrial fibrosis and study outcome, OR or HR, 95% CI
Akoum et al, 2011 (132)	144	283±167 days	DE-MRI	AF catheter ablation	AF recurrence	Increasing recurrence rate with increasing fibrosis degree
Canpolat et al, 2015 (133)	41	18 months	DE-MRI, TGF-β1	AF catheter ablation	AF recurrence	1.127
den Uijl et al, 2011 (134)	170	12±3 months	IBS	AF catheter ablation	AF recurrence	2.80 (2.17-3.61)
Ho et al, 2014 (135)	3306	10 years	Galectin-3	NA	Incident AF	1.19 (1.05-1.36)*
Kainuma et al, 2011 (136)	24	NA	Histology	MAZE procedure, mitral valve surgery	Unsuccessful MAZE procedure	25.2 (1.1-567)
Kallergis et al, 2014 (137)	164	2 months	CITP	DC cardioversion	AF recurrence	3.25

Kawamura et al, 2012 (138)	142	2 years	PIIINP	Pharmacologic or DC cardioversion	AF recurrence	2.63 (1.32–3.56)
Kornej et al, 2015 (139)	119	6 months	Galectin 3	AF catheter ablation	AF recurrence	Higher in AF patients but did not predict AF recurrence
Kubota et al, 2012 (140)	27	3 years	IBS	None	Progression from paroxysmal to persistent AF	HR 8.74 for patients with IBS ≥ 20 dB vs < 20 dB
Kuppahally et al, 2010 (141)	68	1 year	DE-MRI	AF catheter ablation	AF recurrence	1.04 (1.01-1.08)
Ling et al, 2014 (142)	132	1, 3, 6, 12, 18, 24, 30 months	T1 mapping	AF catheter ablation	AF recurrence	38% of AF recurrence in patients with T1 time <230 ms vs 25% in patients with T1 time >230 ms
Malcolme-Lawes et al, 2013 (143)	50	1 year	DE-MRI	AF catheter ablation	AF recurrence	Increasing recurrence rate with increasing fibrosis degree

Marrouche et al, 2014 (144)	272	475 days	DE-MRI	AF catheter ablation	AF recurrence	1.06 (1.03-1.08)
McGann et al, 2014 (145)	386	1 year	DE-MRI	AF catheter ablation	AF recurrence	4.89
Oakes et al, 2009 (146)	81	9.6±3.7 months	DE-MRI	AF catheter ablation	AF recurrence	4.88 (1.73–13.74)
Olasinska-Wisniewska, 2012 (147)	66	12 months	Histology	AF catheter ablation, mitral valve surgery	AF recurrence	1.09 (1.012-1.17)
Park et al, 2013 (148)	128	1 year	TGF-β1	MAZE procedure	Absence of atrial mechanical contraction	7.47 (1.63-34.4)
Rienstra et al, 2014 (149)	3217	10 years	Soluble ST2	NA	Incident AF	No association
Rosenberg et al, 2014 (150)	2935	8.8 years	PIIINP	NA	Incident AF	0.85 (0.72-1.00) and 0.93 (0.88-0.99) at the 10^{th} and 25^{th} percentiles, setting the median

						as the reference, no association at the 75th and 90th percentiles
Sasaki et al, 2014 (76)	113	13.8 (8.7–19.9) months	IBS	AF catheter ablation	AF recurrence	1.04 (1.01-1.07)
Seitz et al, 2011 (151)	22	NA	DE-MRI	AF catheter ablation	'Difficulty' of AF ablation (time to terminate AF; radiofrequency duration until AF termination; complex fractionated atrial electrograms area/LA surface)	Significant correlation between the fibrosis grade and the electrophysiological substrate indexes
Wang et al, 2009 (152)	74	NA	IBS	CABG	Postoperative AF	Higher IBS in postoperative AF versus SR
Wang et al, 2012 (153)	80	6 months	Histology, TGF-β1	Modified MAZE procedure, mitral valve	AF recurrence, absence of atrial mechanical	Increasing recurrence rate with increasing TGF-β1 expression

				surgery	contraction	
Wu et al, 2013 (124)	200	10.9±7.4 months	TGF-β1	AF catheter ablation	AF recurrence	1.11 (1.01–1.22)

* Significant association in univariate analysis only, not significant after adjustment for traditional clinical AF risk factors

AF, atrial fibrillation; CABG, coronary artery bypass graft; CI, confidence interval; DE-MRI, delayed enhancement magnetic resonance imaging; HR, hazard ratio; IBS, integrated backscatter; NA, not available; OR, odds ratio; other abbreviations as in Table 1.

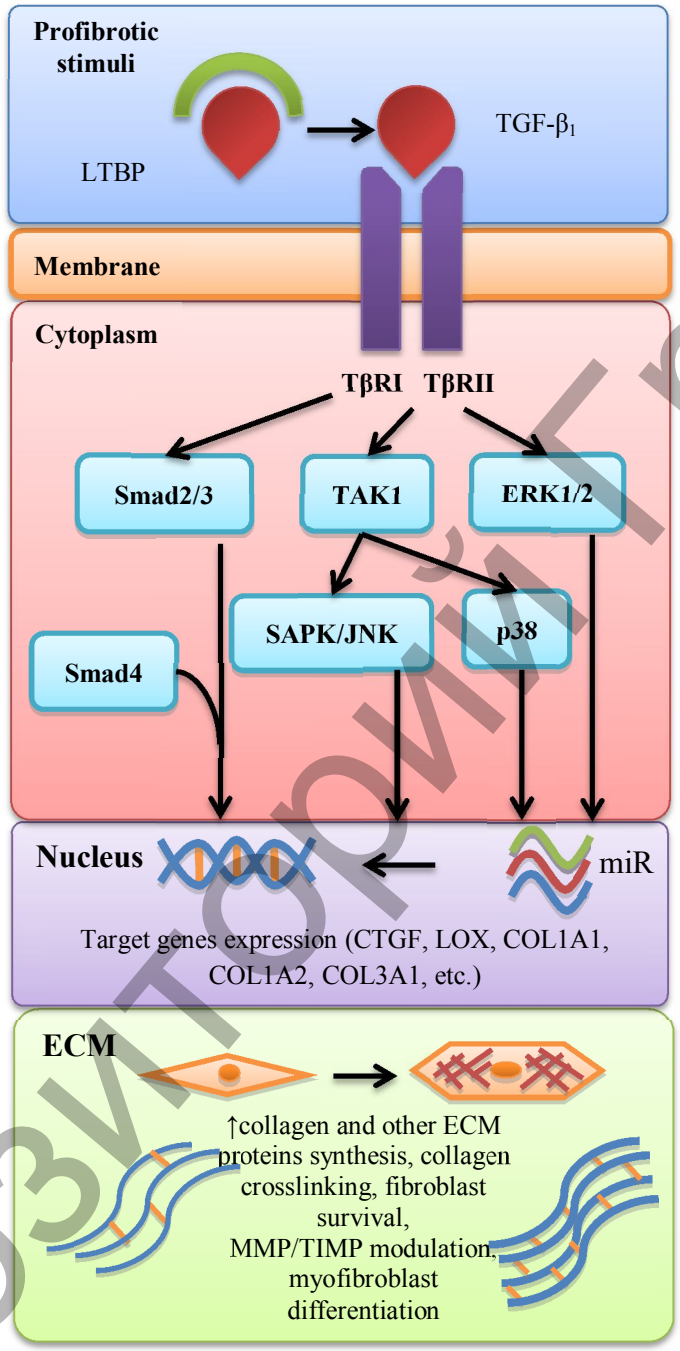

Figure 1. Schematic overview of the TGF-β1 signaling pathway in cardiac fibrosis.

COL1A1, gene encoding α1 type I collagen; COL1A2, gene encoding α2 type I collagen; COL3A1, gene encoding α1 type III collagen; CTGF, connective tissue growth factor; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; JNK, c-jun N-terminal kinase; LOX, lysyl oxidase; LTBP, latent TGF-β1 binding protein; MMP, matrix metalloproteinase; p38, protein 38 (member of MAPK, mitogen-activated protein kinases); SAPK, stress-activated protein kinase; Smad, transcriptional factor (see Table 1); TAK1, TGF-β1 activated kinase 1; TβRI, type I TGF-β1 receptor; TβRII, type II TGF-β1 receptor; TGF-β1, transforming growth factor β1; TIMP, tissue inhibitor of MMP.

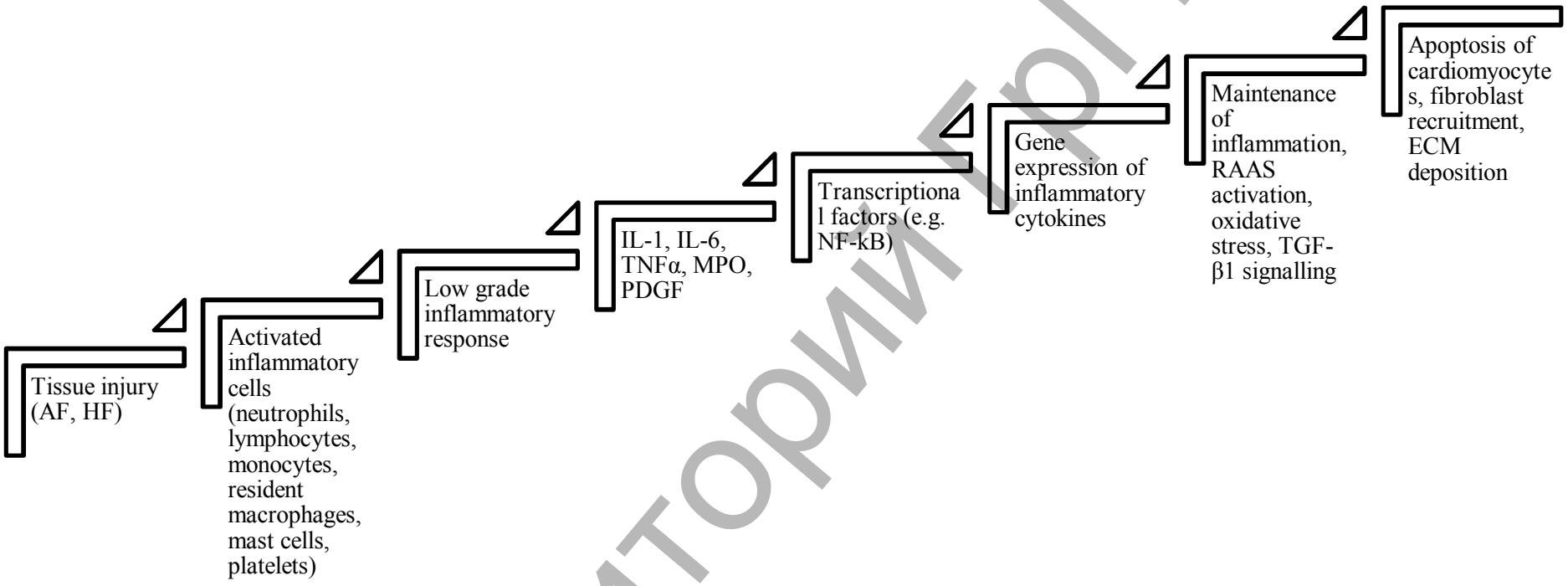

Figure 2. Involvement of inflammation pathways in cardiac fibrosis

AF, atrial fibrillation; ECM, extracellular matrix; HF, heart failure; IL, interleukin; MPO, myeloperoxidase; NFκB, nuclear factor kappa B; PDGF, platelet derived growth factor; RAAS, renin angiotensin aldosterone system; TNFα, tumor necrosis factor α

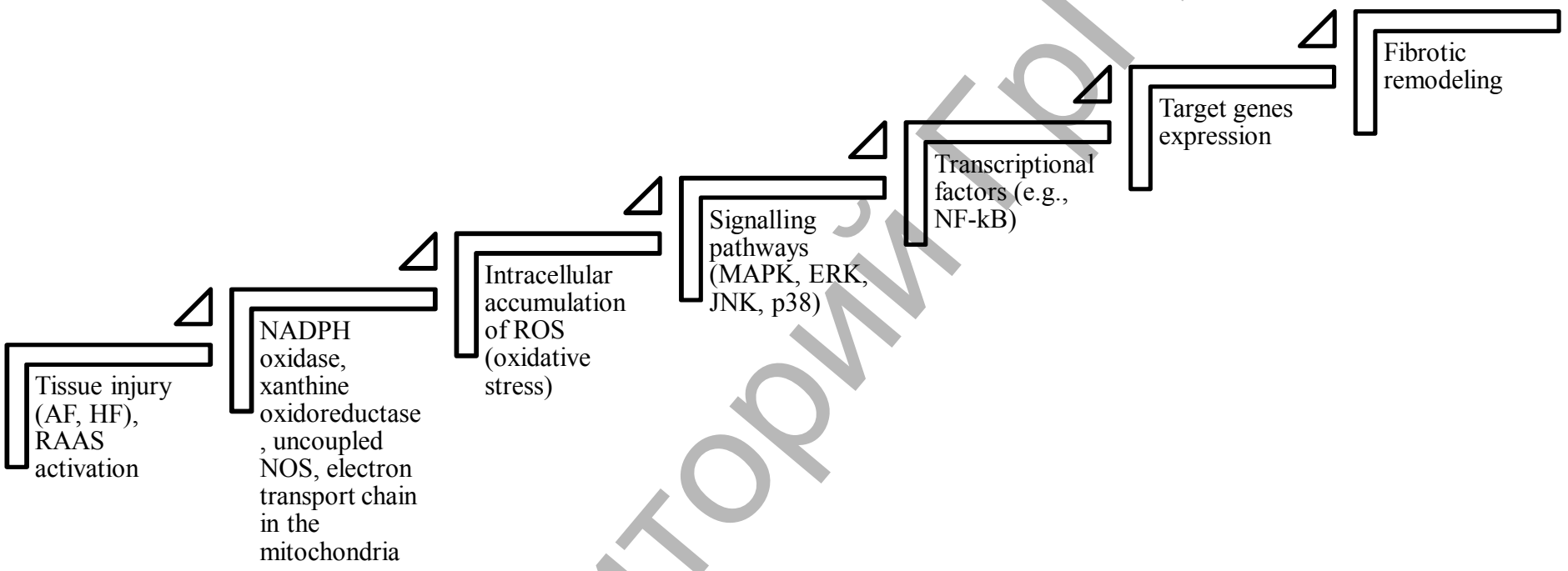

Figure 3. Oxidative stress in cardiac fibrosis

NADPH, reduced nicotinamide-adenine dinucleotide phosphate; MAPK, mitogen-activated protein kinase; p38, protein 38 (member of MAPK, mitogen-activated protein kinases); ROS, reactive oxygen species; other abbreviations as in Figures 1 to 2.

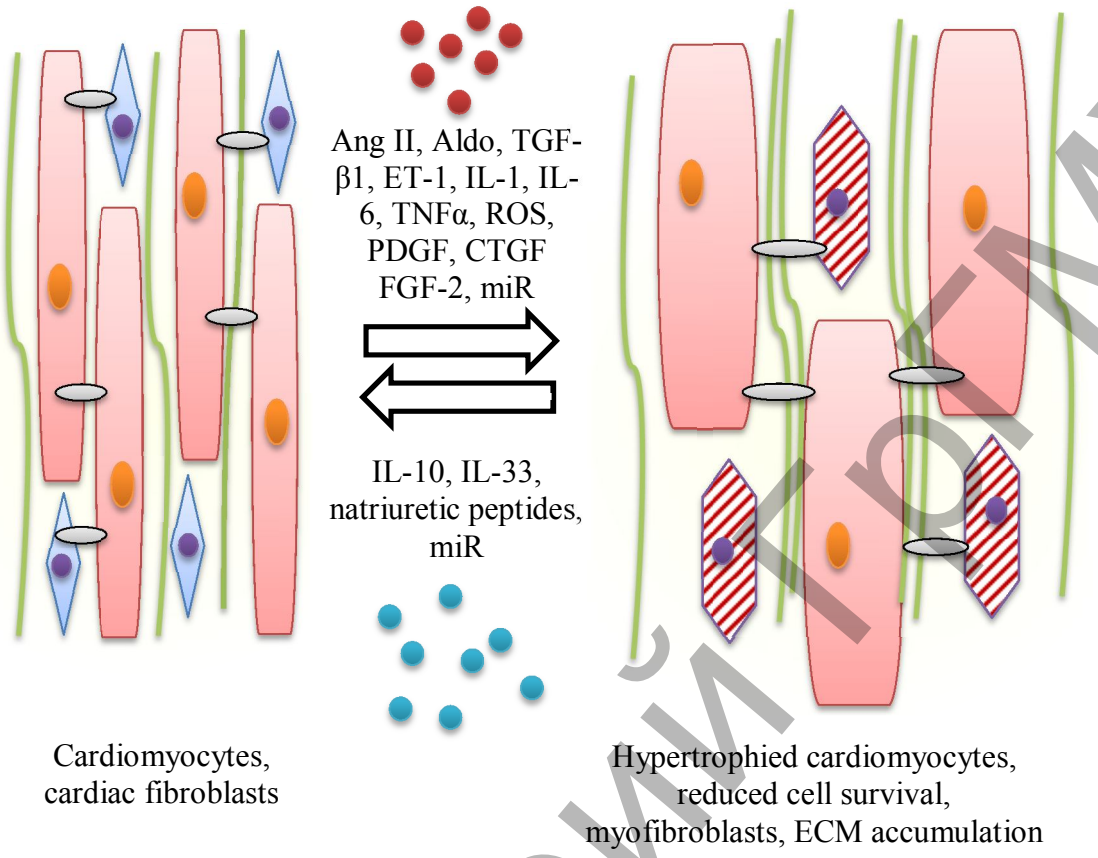

Figure 4. Cardiomyocytes, cardiac fibroblasts and myofibroblasts crosstalk and paracrine factors mediating profibrotic and antifibrotic effects

Aldo, aldosterone; Ang II, angiotensin II; ET-1, endothelin-1; FGF-2, fibroblast growth facor 2; IL, interleukin; miR, microRNA; other abbreviations as in Figures 1 to 3.