Atrial fibrosis underlying atrial fibrillation (Review)

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Abstract. Atrial fibrillation (AF) is one of the most common tachyarrhythmias observed in the clinic and is characterized by structural and electrical remodelling. Atrial fibrosis, an emblem of atrial structural remodelling, is a complex multifactorial and patient-specific process involved in the occurrence and maintenance of AF. Whilst there is already considerable knowledge regarding the association between AF and fibrosis, this process is extremely complex, involving intricate neurohumoral and cellular and molecular interactions, and it is not limited to the atrium. Current technological advances have made the non-invasive evaluation of fibrosis in the atria and ventricles possible, facilitating the selection of patient-specific ablation strategies and upstream treatment regimens. An improved understanding of the mechanisms and roles of fibrosis in the context of AF is of great clinical significance for the development of treatment strategies targeting the fibrous region. In the present review, a focus was placed on the atrial fibrosis underlying AF, outlining its role in the occurrence and perpetuation of AF, by reviewing recent evaluations and potential treatment strategies targeting areas of fibrosis, with the aim of providing a novel perspective on the management and prevention of AF.

Contents

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1. Introduction

Atrial fibrillation (AF) decreases the quality of life of patients whilst also presenting as a financial burden, due to its severe complications (1). Although significant progress has been made in the treatment options that are available for management of AF, such as drugs and catheter ablation, there are complications associated with these strategies, and they are hampered by low long-term success rates. An improved understanding of the fundamental mechanisms underlying the development of AF and subsequent atrial remodelling may facilitate the development of novel and more effective therapeutic approaches for AF treatment. However, the mechanisms underlying AF are complex, and include structural and electrical remodelling, autonomic nervous system dysfunction (2) and dysregulated calcium homeostasis/handing (3). Atrial structural remodelling is the key factor linking all the AF-related mechanisms, and atrial fibrosis is the most prominent feature of atrial structural remodelling (4), but an in-depth understanding of the molecular mechanisms underlying this process has not yet been fully elucidated. For this reason, in the present review, the body of knowledge regarding AF pathophysiology, as well as the involvement of atrial fibrosis in the initiation and perpetuation of AF, were reviewed, and the available fibrosis-guided approaches for prevention and management of AF are discussed (Fig. 1).

As mentioned above, atrial fibrosis is an hallmark of atrial structural remodelling, characterized by the aberrant activation, proliferation and differentiation of fibroblasts, and subsequent excessive synthesis and irregular deposition of extracellular matrix (ECM) proteins, which have been identified as substrates of AF, and are involved in the initiation and perpetuation of AF (5). Atrial fibrosis can be divided into two types, reactive and reparative fibrosis (6,7). Reactive fibrosis is a response to cardiac inflammation or pressure overload, and can be divided into perivascular and interstitial fibrosis (8). Reparative fibrosis occurs due to the loss of cardiomyocytes, with myocardial infarction being the most cause (8). Various pro-fibrotic stimulants activate

fibroblasts to proliferate and differentiate into secretory myofibroblasts, often accompanied by the upregulation of matrix metalloproteinases (MMPs) and downregulation of tissue inhibitors of metalloproteinases (TIMPs). These abnormalities result in an imbalance in ECM deposition and degradation in the intervascular space and myocardial interstitium, ultimately altering the cardiac ultrastructure (8,9). The primary benefit of fibrosis is to maintain the integrity of the heart. However, these collagen-based scars can form barriers to electrical conduction and separate the well-connected syncytium, thereby directly interfering with conduction (10). In addition to physical uncoupling, the membrane of fibroblasts and myofibroblasts can fuse with that of cardiomyocytes to form gap junctions via connexins 40, 43 and 45 (Cx40, Cx43 and Cx45) (11,12). Despite the passive electrophysiological qualities of fibroblasts and myofibroblasts, they have a lower membrane potential than atrial resting potential and can act as an electrical source during their resting phase and as a sink during their activation, thereby reducing the conduction speed and maximum level of depolarization of action potentials (13). It has also been reported that cross-linked collagen between cardiomyocyte bundles forms a thick insulating layer that increases longitudinal conduction velocity, which is also associated with the occurrence of AF (14). When sufficient fibroblasts/myofibroblasts-cardiomyocytes interactions are formed, the arrhythmogenic mechanisms are fulfilled (15). Pathological coupling escalates the spontaneous depolarization during phase 4, and this favours triggered activity (15). Anatomical barriers decrease conduction velocity and increase conduction heterogeneity, as well as the dispersion of refractoriness, which favours re-entry (13). The interactions between triggered activity and arrhythmogenic substrates allows for the occurrence and perpetuation of AF (Fig. 2).

2. Cardiac fibroblasts, myofibroblasts and ECM

In total, four types of cells, namely endothelial cells, cardiomyocytes, fibroblasts and smooth muscle cells, make up a large proportion of cardiac cells (16). Fibroblasts are the second largest population of non-myocyte cells in the heart, accounting for ~10% of cardiac cells, and are the primary source of ECM (17). Cardiomyocytes are predominant in volume, and are the primary constituents of the heart (18). The distribution of cardiac fibroblasts in the atrium is higher than that in the ventricles, and the responses of atrial fibroblasts to pro-fibrotic stimuli are different from those of ventricular fibroblasts, which may account for the difference in the degree of fibrosis between atriums and ventricles under similar pathological conditions (19,20).

Cardiac fibroblasts are flat, spindle-shaped cells that are generally considered to have a mesenchymal origin, and they determine the homeostasis of ECM (21). During the development of the heart, most cardiac fibroblasts are differentiated from epicardium-derived cells (22). The rest are derived from the endocardium and the neural crest, which are primarily located in the interventricular septum and right atrium, respectively (Fig. 2) (23,24). Under homeostatic conditions, fibroblasts remain dormant. Apart from the activation and proliferation of resident fibroblasts, several cell linages, such as endothelial cells, bone marrow progenitor cells, circulating fibrocytes and monocytes, can differentiate into fibroblasts when activated by pathological stimulants, thus, markedly increasing the number of cardiac fibroblasts (25,26). Activated fibroblasts then synthesize not only a variety of ECM proteins, but also proteolytic enzymes that modify these proteins and can differentiate into myofibroblasts, which are contractile cells with a more potent ability to synthesize more ECM proteins (27). This differentiation causes disequilibrium in the synthesis and degradation of ECM proteins, ultimately leading to an arrhythmogenic atrial substrate (28).

The ECM not only acts as a scaffold for all cells in the heart, but it is also involved in regulating cardiac function and mediating extracellular signal transmission (29). In addition to collagens, proteoglycans, glycoprotein and other proteins (such as MMPs and TIMPs) are necessary components of the ECM (30). There are also non-glycosylated proteins and soluble components within the extracellular space, such as dermatopontin, transforming growth factor β (TGF-β) and interleukins (ILs), which are involved in the regulation of ECM remodelling (31). Of these, collagens (primarily types I and III) are the predominant constituents of the cardiac ECM (5). The synthesis of collagens starts when their progenitors, pro-collagens, are cleaved by procollagen C-terminal proteinase and procollagen N-terminal proteinase at the C- and N-terminal domains to form mature collagen molecules (9). The final step is self-assembly and cross-linking of mature collagen molecules form collagen fibres. In this enzymatic process, some proteolytic products, such as N-terminal pro-peptide of procollagen type III and C-terminal pro-peptide of procollagen type I, are released into the blood and can be used as biomarkers to assess cardiac fibrosis and evaluate AF recurrence (32-34). The process of ECM protein synthesis is a dynamic and balanced process under the fine regulation of proteolytic enzymes and their inhibitors (9). Amongst these enzymes, the most important are the MMP family members of which there are >25. They can not only degrade almost all ECM proteins, but also cytokines and growth factors, amongst other molecules, which affects the synthesis of ECM (35). Increased expression of MMP-9 has been observed in the atrial tissue and blood serum of patients with AF, and the MMP-9 levels appear to be associated with the stage of AF (36,37). In addition, it was found that serum MMP-9 levels can also be used as an independent factor to predict the recurrence of AF following catheter ablation (38). A previous meta-analysis demonstrated that the enhanced MMP-1 mRNA expression and decreased serum TIMP-2 levels may act as predictive markers for the incidence of AF (39). In addition, MMP-2 was also shown to be associated with an increased risk of AF, and may be used to identify patients that are most likely to benefit from rhythmic control strategies (40).

3. Risk factors involved in atrial fibrosis

The past few years have witnessed an impressive growth in the number of studies studying the signalling pathways involved in atrial fibrosis, but the specific mechanism remains poorly understood. However, some effective therapeutic options that

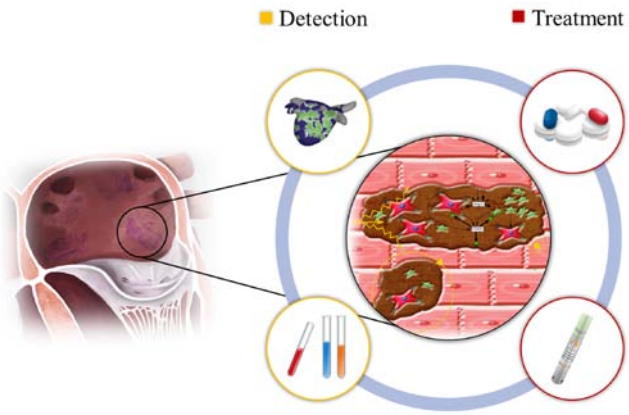

Figure 1. Mechanism by which atrial fibrosis causes atrial fibrillation and the methods for diagnosis and treatment of atrial fibrosis.

target atrial fibrosis could not have been developed without taking into account the complex signalling pathways. The key factors and mechanisms leading to progressive atrial fibrosis are discussed below (Fig. 3).

TGF-β_1. TGF-β is one of the most potent pro-fibrotic growth factors, with >30 family members, including TGF-β_{1-3}, of which TGF-β_1 is the predominant member (41). TGF-β_1 promotes the synthesis of collagen fibres by cardiac fibroblasts and their differentiation into myofibroblasts via the typical Smad-dependent and non-canonical Smad-independent pathways (42). In the canonical Smad-dependent pathway, TGF-β binds to two types of serine/threonine kinase receptors [type I TGFβ receptor (TβRI)/activin receptor-like kinase 5 and TβRII], which together form a Smad2/3/4 complex that subsequently leads to Smad protein-mediated signal transduction (43,44). Smad7, an inhibitory Smad, antagonizes the TGF-β/Smad signalling pathway (44). Non-canonical pathways include the mitogen-activated protein kinases (MAPKs)/TGF-β_1/tumour necrosis factor (TNF) receptor associated factor 6/TGF-β-activated kinase 1, TGF-β_1/cluster of differentiation (CD)44/signal transducer and activator of transcription 3 (STAT3) and angiotensin II (Ang II)/TGF-β/Ras homolog family member A (RhoA)/Rho-kinase (ROCK) signalling pathways (45-47). The thrombospondin-1/TGF-β/MMP-9 axis is also involved in atrial fibrosis in patients with AF (48).

Atrial myofibril loss was higher in patients with AF compared with those with sinus rhythm. In an electrical stimulation experiment of cultured HL-1 atrial myocytes, Yeh *et al* (49) demonstrated that Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-mediated oxidative stress may account for tachycardia-induced myofibril degradation. They also reported increased levels of p-Smad3 in a tachypacing model, and confirmed there was crosstalk between the two signalling pathways in tachypacing-stimulated reactive oxygen species (ROS) production.

Renin-Ang-aldosterone system (RAAS). The RAAS is a system involving the pathophysiological involvement of multiple organs including the heart, kidney and lungs (50). Ang II is a major mediator of this system and serves an important role in atrial fibrosis. Ang II exerts pro-fibrotic effects by binding to its type 1 receptor (AT_1-R), a member of the G-protein-coupled receptor superfamily. G protein activation stimulates phospholipase C (PLC) to generate inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 mediates the increase of Ca^{2+} levels in the cytoplasm. Intracellular Ca^{2+} overload promotes fibroblast proliferation and differentiation (51). DAG activates protein kinase C, which in turn activates extracellular-signal-regulated kinases (ERKs). In addition, acting as a potent NADPH oxidase activator, Ang II induces ROS overproduction, which, in-turn, activates multiple downstream second messengers, including MAPK, nuclear factor-κB and cytokines (52,53). Through the activation of the MAPK signalling pathway, Ang II promotes the secretion of TGF-β_1. TGF-β_1 reciprocally upregulates the density of AT_1-R and the expression of connective tissue growth factor (CTGF), thereby further promoting fibrosis (54). Conversely, the stimulation of Ang II type 2 receptor (AT_2-R) constrains the pro-fibrotic effects of AT_1-R (55). Several studies have confirmed that the blockade of Ang II by Ang-converting enzyme inhibitors (ACEIs) or Ang receptor blockers (ARBs) reduces atrial fibrosis (56,57). Aldosterone is the end product of RAAS, and its role in AF pathophysiology has proven very valuable. By binding to the mineralocorticoid receptor (MR), aldosterone serves its pro-fibrotic roles via the MAPK intracellular signalling pathway in HL-1 atrial myocytes (58,59). Furthermore, there is crosstalk between the MR/AT_1-R and MAPK signalling pathway, suggesting that the combined blocking of MR and AT_1-R can prevent the occurrence of AF (59).

Inflammation. A previous study suggested that inflammation is closely associated with AF (60). This association was first noticed due to the high incidence of postoperative AF (60). Bruins *et al* (60) first reported an association between C-reactive protein and arrhythmia in patients who suffered from coronary artery disease. Inflammatory cell infiltration and an increased serum level of inflammatory mediators, such as IL-1β, IL-6, IL-8, IL-10 and TNF-α were found to be associated with AF. Not only do the expression levels of these inflammatory mediators increase as the duration of AF increases, but some of these mediators can even be used to predict postoperative AF recurrence (61).

The pro-fibrotic effect of inflammation is generally attributed to oxidative stress, which promotes the initiation and perpetuation of AF by activating the MAPK signalling pathway (62). Mitochondria and NADPH oxidase are hypothesized to be the major sources of ROS, which is a second messenger that activates downstream signals. Amongst other things, uncoupled nitric oxide (NO) synthase and xanthine oxidase are also sources of ROS (63). Ang II promotes ROS production, and both are involved in aberrant Ca^{2+} handling, increasing the cytosolic Ca^{2+} concentration (64,65). Intracellular Ca^{2+} overload further aggravates electrical remodelling by downregulating the L-type Ca^{2+}-current (66). In addition, microRNA (miRNA/miR)-26 is also downregulated by the activation of the Ca^{2+}-calcineurin-nuclear factor of activated T-cells signalling pathway, promoting the expression of KCNJ2/I_{K1} in both cardiomyocytes and fibroblasts. Treatments targeting the upstream inflammatory cascade can decrease the inflammatory response and oxidative stress, and

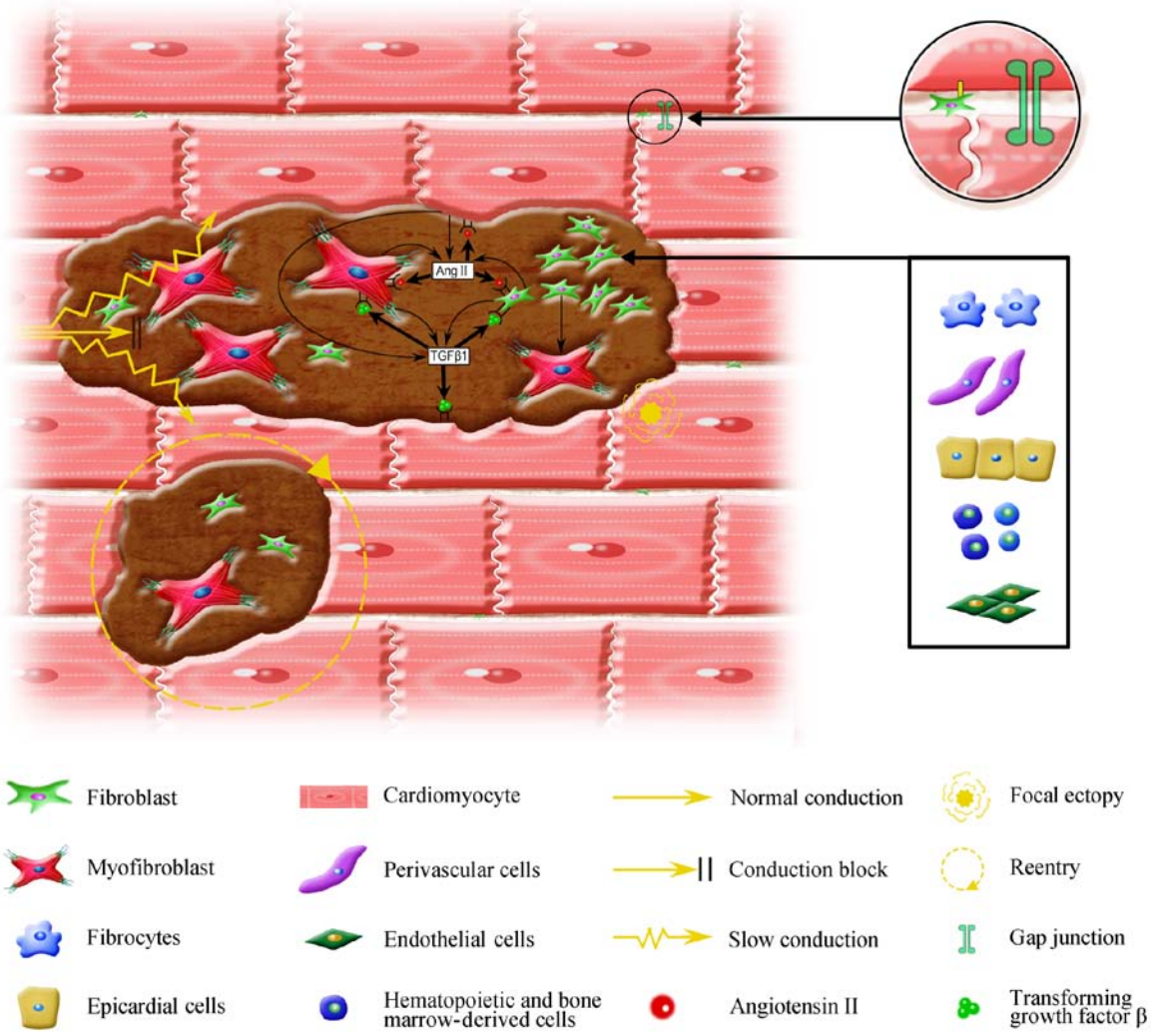

Figure 2. Occurrence and perpetuation of atrial fibrillation and the origins of cardiac fibroblasts.

alleviate atrial structural and electrical remodelling, which further elucidates the mechanisms underlying this disease (67).

Adipose, particularly epicardial adipose tissue (EAT), is strongly associated with the initiation, duration and recurrence of AF. With regard to the pathological mechanism of EAT by which it promotes the occurrence and development of atrial fibrosis, considerable evidence has consistently confirmed its role in local inflammation. Abe *et al* (68,69) evaluated the levels of cytokines/chemokines in a specimen from human left atrial appendage. The results showed that the expression levels of IL-1, IL-6, IL-10 and TNF-α in EAT increased, consistent with a previous result from Mazurek *et al* (68,69). In addition, adipokines secreted by EAT are another mechanism underlying fibrosis. Activin A, an adipokine belonging to the TGF-β superfamily, has the ability to initiate atrial fibrosis (70). In addition, CTGF, a fibrotic cytokine that functions via the TGF-β_1/Smad pathway, has been shown to be upregulated in EAT and is strongly associated with AF (71,72).

Platelet-derived growth factor (PDGF). PDGF is a member of the PDGF/vascular endothelial growth factor family, which includes four isoforms, namely, PDGF-A, PDGF-B, PDGF-C and PDGF-D. PDGF serves a role in promoting fibroblast proliferation and differentiation via the MAPK, Janus kinase (JAK)/STAT, Ras/ERK kinase 1/2 and PLC pathways that are shared by both TGF-β_1 and Ang II. Mast cell infiltration and over-synthesis of PDGF-A were observed in mice atria affected by cardiac pressure overload, and atrial fibrosis and susceptibility to AF were increased in these mice. A PDGF-A-targeting antibody, as well as mast cell stabilizer or genetic mast cell depletion can attenuate these changes (73). Chen *et al* (74) evaluated the potential role of the PDGF-JAK-STAT pathway in LA-remodelling using a ventricular tachypacing-induced canine congestive heart failure (CHF) model. It was observed that the overexpression of PDGF-A, -C and -D in LA fibroblasts of HF canine enhanced JAK-STAT expression and ECM secretion. Furthermore, the high levels of these PDGF isoforms substantially upregulated the mRNA expression levels of TGFβ_1, which, in turn, advanced cardiac fibrosis (75).

MiRNAs. In vivo and *in vitro* studies have suggested that miRNAs may also serve a role in atrial fibrosis and AF. In addition to being involved in electrical remodelling, miRNAs also play important roles in atrial structural remodelling. Li *et al* (76) showed that miR-10a could inhibit the TGF-β_1/Smad signalling pathway to decrease the synthesis of

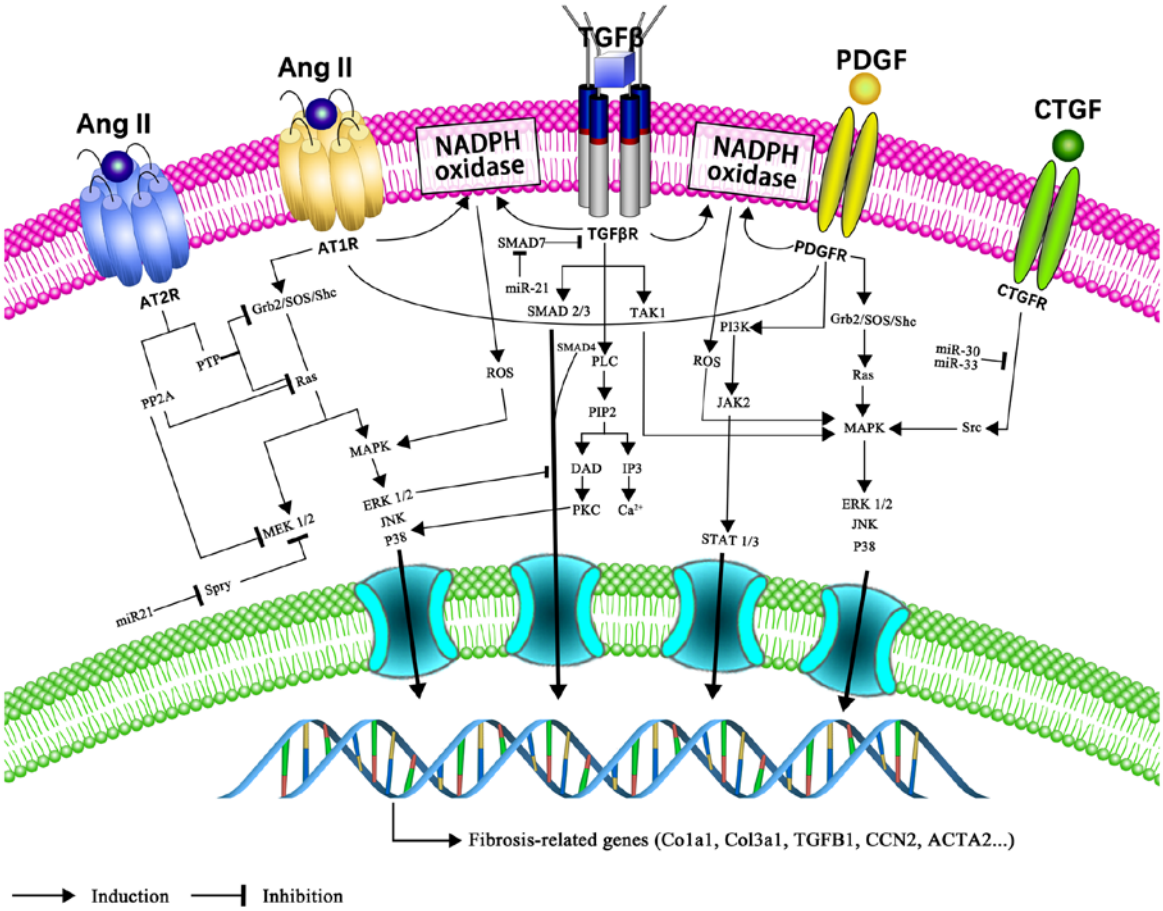

Figure 3. Signalling pathways associated with atrial fibrosis.

collagen, suppress the proliferation of cardiac fibroblasts and ameliorate cardiac fibrosis. Studies have shown that miR-21 can target sprouty homolog 1, an ERK inhibitor, which activates the ERK/MAPK signalling pathway to promote cardiac fibroblast proliferation and fibrogenesis (77,78). A previous study by He *et al* (79) demonstrated that Smad7 is also a target of miR-21. They used rapid atrial pacing in rats to induce atrial fibrosis and AF. The results showed a higher expression level of miR-21 and lower levels of Smad-7, blunting the inhibitory effect of Smad7 on the TGF-β/Smad-2/3 signalling pathway (79). In a study by Wang *et al* (80) miR-27b was found to inactivate the Smad2/3 pathway, reducing the incidence and duration of AF, as well as attenuating atrial fibrosis, which was evidenced by the reduced expression levels of smooth muscle α-actin, collagen-I and collagen III (80). miR-30 and miR-133 target TGF-β and TGF-β receptor to affect collagen synthesis (81). They can also negatively regulate cardiac fibrosis by inhibiting the expression of CTGF.

4. Ventricular fibrosis in AF

Significant non-invasive technological advances have opened up more possibilities for the characterization and quantification of focal and diffuse left ventricular (LV) myocardial fibrosis in patients with AF, which have provided evidence that the cardiac pro-fibrotic microenvironment in AF is unlikely to be strictly limited to the atria (82,83). Late gadolinium enhanced cardiac magnetic resonance (LGE-CMR) imaging is an established technique for the evaluation of focal myocardial scars on the basis of the different abilities of healthy myocardium and areas of fibrotic tissue to clear gadolinium (84). With regard to diffuse myocardial fibrosis, gadolinium contrast may be evenly retained throughout the diffusely fibrotic myocardium, and the signal intensity of diffusely fibrotic areas may be nearly isointense, as compared with that of normal tissue. Diffuse interstitial fibrosis is challenging to distinguish using conventional delayed enhancement (DE)-CMR (85-87). With the development of novel contrast-enhanced T_1 mapping techniques, diffuse myocardial fibrosis may be detected through a quantitative measure of the myocardial T_1 relaxation times (86,87). Ling *et al* (88) used myocardial T_1 mapping in patients with AF to detect diffuse myocardial fibrosis of the LV. They showed that LV fibrosis could be detected and quantified by T_1 mapping in patients with AF and HF concurrently. Of note, several studies have shown that diffuse ventricular fibrosis measured by T1 mapping on CMR predicts the success of catheter ablation for AF, although the mechanism behind this association is not clear (89,90).

There may be some possible explanations for the association between AF and the presence of diffuse LV fibrosis. For example, arrhythmia-mediated cardiomyopathy may predispose patients to diffuse interstitial fibrosis (91). Ventricular fibrotic changes are more extensive in patients with AF compared to those with sinus rhythm (82,92). Data from an animal study suggested that a rapid ventricular response from AF could result in a decrease in ventricular function, and an

increase in ventricular and atrial fibrosis (93). In addition, the restoration of the sinus rhythm with catheter ablation is accompanied by significant improvements in reverse cardiac remodelling and ventricular function (94). Fibrotic cardiomyopathy has been suggested to predispose patients to diffuse interstitial fibrosis development. A plethora of non-cardiac factors have been shown to contribute to fibrosis in AF, including obesity, systemic inflammation, metabolic syndrome, thyrotoxicosis and obstructive sleep apnoea, which could ultimately affect the myocardium (95). Obstructive and central sleep apnoea leads to myocardial hypertrophy and diastolic dysfunction, thus further potentiating the development of HF in patients with AF (96,97). Obesity in AF is associated with diastolic ventricular impairment and myocardial lipidosis (98). Alternatively, the association between AF and ventricular fibrosis may also be due to other factors which have yet to be uncovered (99). In summary, ventricular fibrosis in response to AF may be regulated by multiple mechanisms. Additional studies focusing on the association between AF and diffuse myocardial fibrosis are required.

Several common mechanisms are known to contribute to atrial and ventricular fibrosis in AF, whereas the extent of fibrosis may vary between the 2 parts of the heart. Transgenic mice with TGF-β_1 exhibited higher TGF-β_1 levels in the atria than in the ventricles under the control of an α-MHC promoter (100). In this model, 80 pro-fibrotic genes in the atria were overexpressed and only 2 genes in the ventricle were differentially expressed, as shown by RNA microarray analysis (100). Similarly, transgenic mice overexpressing ACE exhibited a hypertrophic and dilated atria with focal atrial fibrosis, but normal ventricles (101). This differential chamber-specific fibrotic response to ACE overexpression could be partly explained by the differential AT_1 receptor expression in the atria and ventricles (102). It has been shown that atrial fibroblasts show greater fibrotic and oxidative responses to TGF-β_1 than ventricular fibroblasts (103), indicating that the atria has a more potent fibrotic response to various stimuli (20). The results of these studies suggested that the mechanisms involved in the development of atrial and ventricular fibrosis are different. Further studies are required to investigate whether other important signalling pathways contribute to the development of selective fibrosis in the atria, compared to the ventricles.

5. Atrial fibrosis and stroke risk in AF

There is increasing evidence of an association between atrial fibrosis and the risk of stroke in patients with AF. Daccaret *et al* (104) identified an association between the percentage of atrial fibrosis detected on LGE-CMR and a higher $CHADS_2$-score [CHF, hypertension, age >75 years, diabetes mellitus and stroke or transient ischemic attack (TIA)], and a history for stroke. Left atrium fibrosis is a strong predictor of left atrial thrombosis or cerebrovascular events, particularly stroke or TIA (105,106). Another study by Disertori *et al* (107) showed that the risk of stroke may be independently associated with structural fibrotic remodelling. Left atrial fibrosis is also associated with an increased risk of cryptogenic stroke (108). Even in patients without AF, embolic stroke of an undetermined source has been found to be correlated to atrial fibrosis (109). Spronk *et al* showed that hypercoagulability in itself may stimulate fibroblasts and increase fibrosis. It was revealed that anticoagulation therapy may prevent thromboembolic events, partly through influencing the substrate by reducing the degree of fibrosis (110). In combination, these studies provided quantitative evidence that the risk of stroke in patients with AF may be associated with the severity of the LA fibrosis. However, there is a paucity of data on the pathophysiological link and molecular mechanisms between atrial fibrosis and thromboembolism. Atrial fibrosis, one of several markers of an AF-prone atrial substrate, promotes the re-entry of electrical current by increasing heterogeneity of conduction in the atria, which ultimately impairs atrial contractility, and reduces ejection fraction and flow velocity (111). It thus causes increased platelet aggregation, which further enhances the milieu of intra-atrial stasis (111). Endothelium/endocardial tissue not only forms a barrier between platelets and extracellular matrix, but also secretes factors such as NO and heparan sulphates to prevent the activation of the coagulation cascade. Endothelial dysfunction develops as a result of atrial fibrosis in patients with AF and promotes thrombus formation (112).

Inflammation and oxidative stress are known to serve an important pathogenic role in AF, leading to cardiac fibrosis (113,114). Inflammatory markers, such as TGF-β1, IL-6 and TNF-α, have been detected in patients with AF and have been shown to affect the functional stability of myocytes and endothelial cells, as well as promote atrial fibrosis (53,115). Inflammatory marker levels were associated with a risk of stroke in patients with chronic AF during follow-up (116,117). There may be a close interplay amongst atrial fibrosis, inflammation and oxidative stress, which, in turn, leads to endothelial and/or endocardial dysfunction and a pro-thrombotic state; however, further studies are required to advance from theoretical to pragmatic outcomes.

Stroke in AF appears to be a complex and poorly understood phenomenon, and the means by which LA fibrosis predisposes patients to thrombus formation is not completely clear. LA fibrosis represents a marker of disease, which can improve the prediction of thromboembolic events in patients with AF.

6. Treatment approaches targeting atrial fibrosis

Conventional antiarrhythmic agent approaches have limited efficacy and have several adverse effects. Increased attention has therefore been diverted to upstream therapies with the use of non-antiarrhythmic drugs targeting substrate development and modifying risk factors for human AF. Specifically, one of the most relevant objectives of upstream therapy is the control of the development and progression of atrial fibrosis, which is a hallmark of structural remodelling in AF and is considered a substrate for perpetuation of AF (118-120). It has become clear that Ang II is a potent stimulator of pro-fibrotic pathways during AF, and the inhibition of the RAAS by ACEIs, ARBs and mineralocorticoid receptor antagonists (MRAs) was shown to reduce the progression of fibrosis (121). Several ACEIs have been shown to effectively suppress atrial fibrosis

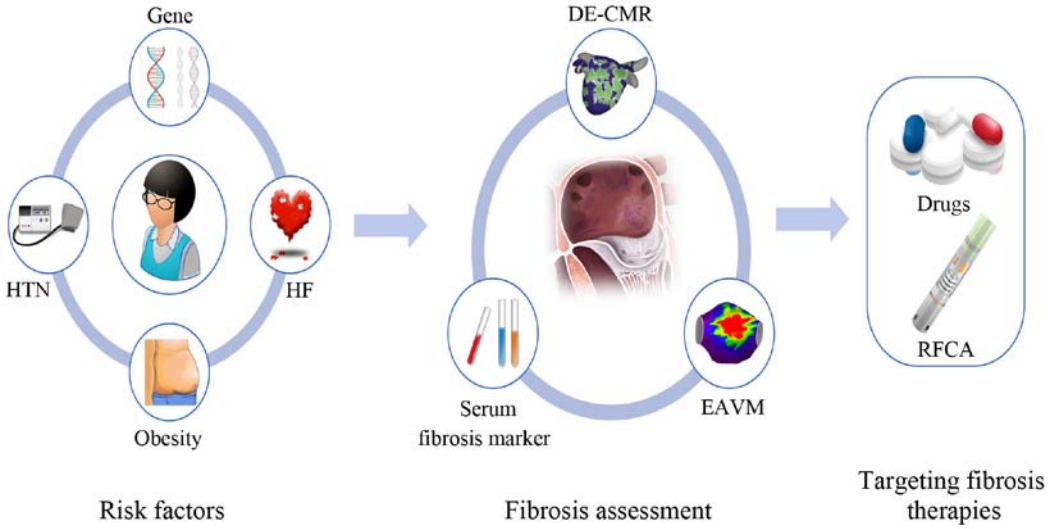

Figure 4. Tailored treatment for atrial fibrosis. HF, heart failure; HTN, hypertension; DE-CMR, delayed-enhancement cardiovascular magnetic resonance; EAVM, electroanatomic voltage mapping; RFCA, radiofrequency catheter ablation.

and prevent the development of the AF substrate (122,123). The potential of AT_1 receptor blockers for the treatment of fibrosis and AF has been previously explored. In spontaneously hypertensive rats, valsartan reduced the degree of myocardial fibrosis (124). Similarly, losartan and candesartan have been previously shown to suppress atrial remodelling by inhibiting left atrial fibrosis and improving AF indices in experimental models (125,126). MRAs also appear to be potential agents for fibrosis. Lavall *et al* (58) found that mineralocorticoid receptor blockers could effectively reduce the incidence of new-onset AF in patients with systolic heart failure. Eplerenone treatment has been shown to inhibit the development of atrial hypertrophy and fibrosis compared with the control group animals (127). Retrospective analyses and meta-analyses of databases from clinical trials have suggested a role of inhibitors of the Ang axis in AF prevention, particularly in patients with LV hypertrophy and systolic LV dysfunction (128-132). However, other clinical studies reported no beneficial effects of Ang blockade treatment on the incidence of recurrent AF (133,134). These conflicting outcomes may be partly attributed to the possible interactions or synergistic effects with other drugs including ACEIs, amiodarone and β-blockers, and the differences in the baseline parameters of patients, such as ventricular function, structural substrates and influence of fibrosis-causing factors. The beneficial effects of upstream therapies may be due to the prevention of structural remodelling in both the left atrium and the LV, improved LV haemodynamics and reduced atrial stretch, and direct or indirect modulation of ion-channel function and other unknown factors.

There is less evidence in favour of therapies, such as polyunsaturated omega-3 fatty acids or the inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (statins) in the inhibition of fibrosis and atrial structural remodelling. Simvastatin attenuated CHF-induced atrial structural remodelling and AF promotion (135). Similarly, statin therapy may contribute to the prevention of AF in the postoperative period of cardiac surgery (136). Omega-3 poly-unsaturated fatty acids have been found to suppress AF in patients with an evident structural substrate and presence of atrial remodelling, combined with high levels of circulating inflammatory biomarkers (137). Treatment of CHF canines with the antifibrotic drug pirfenidone resulted in significantly reduced TGF-β_1 levels, arrhythmogenic atrial remodelling and AF vulnerability (138). In conclusion, these results further highlight the value of upstream AF prevention therapy.

The potential mechanisms underlying the positive effects of atrial fibrosis treatment and any fibrosis-related AF in humans is not well understood. A deeper understanding of these fundamental mechanisms may assist in identifying novel targets for pharmacological interventions, which may be even more effective than conventional antiarrhythmic therapy.

Percutaneous catheter ablation is a widely used and effective clinical treatment for rhythm control in patients with AF (139). Circumferential pulmonary vein isolation (CPVI) alone is an ablation strategy that is effective in the majority of patients with paroxysmal AF. However, the frequent need for re-ablation coupled with the lower long-term success rates are still major limitations of catheter ablation procedures in the treatment of non-paroxysmal AF (140). AF evolves from a singular rhythm disturbance to the complex condition that is cardiomyopathy through arrhythmia substrates (141,142). Studies have reported the detection of atrial fibrosis using DE-MR imaging (MRI) and electroanatomic voltage mapping (EAVM) (104,143-146), and suggested that it is an important predictor of the outcome of AF interventions (146-148).

Substrate modification targeting fibrotic tissue has been performed for several years using EAVM (149); this procedure has been described in more detail previously (149). Kottkamp *et al* (150) described a patient-tailored ablation strategy termed 'box isolation of fibrotic areas', which involves the circumferential isolation of substantially affected fibrotic areas (<0.5 mV), providing a novel selection criterion for PVI-only ablation in patients with non-paroxysmal AF. Rolf *et al* (144) also demonstrated a tailored substrate modification based on voltage criteria. Yamaguchi *et al* (151) described an approach of homogenizing areas of substantial

fibrosis; briefly, the ablation of all detectable electrograms within the target areas was defined as an area with bipolar electrograms of <0.5 mV and, in addition, short linear lesions were created so as to ablate potential conduction channels. During a follow-up in their study, absence of AF was notably higher in the low-voltage zone-based substrate modification group compared with the group that only underwent PVI (38% vs. 72%). A total of 144/201 patients (74%) who underwent LA low voltage area-guided AF substrate modification as an adjunct to PVI during a median follow-up of 3.1 years were free from recurrence (152). Similarly, Jadidi *et al* (153) previously reported that absence of arrhythmia was higher in the substrate modification approach group compared with the matched control group that only received PVI (69% vs. 47%). As compared with the stepwise approach for the treatment of non-paroxysmal AF, a strategy of selective electrophysiologically guided atrial substrate modification after CPVI and cavotricuspid isthmus ablation was found to be more clinically effective (154). Voltage mapping as a tool for describing fibrotic changes remains under investigation and still requires standardization. For example, the measured voltage depends on the rhythm, various thresholds of voltage amplitude used to define fibrotic areas, the contact of the electrode to the tissue, the electrode size and spacing, the thickness of the atrial myocardium and other variables (155).

LGE-CMR provides a non-invasive tool for detecting, quantifying and localizing atrial fibrosis. Jadidi *et al* (156) demonstrated that the large fibrotic substrate detected with LGE-CMR is associated with the complex fractionated atrial electrogram, proposed as a relevant phenomenon maintaining AF. Recent data have reported patients being free of AF recurrence after catheter ablation led to a significant attenuation of the LA fibrosis burden, as shown by follow-up CMR studies (157). In contrast to invasive EAVM during the ablation procedure, LGE-CMR-guided fibrosis management has improved our understanding of the individual underlying arrhythmia substrate during the natural course of human AF. Fochler *et al* (158) reported that an LGE-MRI anatomically guided approach for the treatment of recurrent arrhythmias post-AF ablation is feasible and effective. Similarly, another study highlights the potential use of the optimal set of patient-specific targets to ablate fibrotic atrial substrates (159). The LGE-CMR-guided assessment may provide novel insights into patient-specific AF stages and treatment strategies; however, this modality requires extensive MRI experience, and its reproducibility is still under intensive investigation (160).

At present, the success rate of non-individualized substrate modifications of catheter ablation procedures for patients with persistent and/or long-standing AF is disappointingly low (161). Completely novel catheter ablation strategies that are based on the individual substrates rather than on the 'phenotype' in paroxysmal vs. non-paroxysmal AF are thus required. The knowledge of the individual amount and distribution pattern of a patient's AF fibrotic LA substrate allows for a personalized path to prevention, monitoring or even targeting arrhythmia substrates in patients with AF, which need to be confirmed and validated with respect to efficacy, as well as safety in prospective multicentre randomized studies.

7. Conclusion and future perspectives

The prevalence and health burden of AF worldwide highlights the importance of the development of high-accuracy and precision therapies aimed at preventing or reversing AF. Clinical and experimental studies have reported that atrial fibrosis is closely associated with the occurrence and maintenance of AF. The development of fibrosis is a highly complex, multifactorial and patient-specific process, involved in complex neurohumoral, cellular and molecular interactions. Although a significant understanding has already been obtained that has led to the identification of novel targets for fibrotic mechanism-based therapies, the precise role of fibrosis in AF initiation and maintenance remains to be determined. There is a wide variation in the presence, extent and pattern of LA fibrosis. Its use for AF treatment may assist in designing individually tailored ablation approached for determining the ablation strategy following pulmonary vein isolation and highlights the need for repeated ablation procedures, which could potentially significantly improve our understanding of AF and ablation outcomes.

An improved understanding of the roles, characteristics and mechanisms of fibrosis during AF may facilitate the identification of new clinical biomarkers, as well as assist in the development of novel, more effective and patient-tailored treatment approaches for AF by targeting the fibrotic substrate (Fig. 4).

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Authors' contributions

CYL, JRZ, WNH and SNL wrote the manuscript. SNL critically reviewed the manuscript. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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