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Extracellular Matrix Remodeling in Atrial Fibrosis: Mechanisms and Implications in Atrial Fibrillation

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Abstract

Atrial fibrosis has been strongly associated with the presence of heart diseases/arrhythmias, including congestive heart failure (CHF) and atrial fibrillation (AF). Inducibility of AF as a result of atrial fibrosis has been the subject of intense recent investigation, since it is the most commonly encountered arrhythmia in adults and can substantially increase the risk of premature death. Rhythm and rate control drugs as well as surgical interventions are used as therapies for AF; however, increased attention has been diverted to mineralocorticoid receptor (MR) antagonists including spironolactone as potential therapies for human AF because of their positive effects on reducing atrial fibrosis and associated AF in animal models. Spironolactone has been shown to exert positive effects in human patients with heart failure; however, the mechanisms and effects in human atrial fibrosis and AF remain undetermined. This review will discuss and highlight developments on (i) the relationship between atrial fibrosis and AF, (ii) spironolactone, as a drug targeted to atrial fibrosis and AF, as well as (iii) the distinct and common mechanisms important for regulating atrial and ventricular fibrosis, inclusive of the key extracellular matrix regulatory proteins involved.

Keywords

atrial fibrosis; atrial fibrillation; spironolactone; collagen; extracellular matrix; molecular mechanisms; animal models; mineralocorticoid receptor; ventricular fibrosis

Introduction

Cardiac fibrosis is a detrimental process that causes an imbalance between extracellular matrix (ECM) deposition and degradation within the heart resulting in excessive fibroblast proliferation and build up of ECM proteins within the cardiac interstitial space. To date, ventricular fibrosis has received a great deal of attention in this context since early studies showed it to cause progressive stiffening of the ventricular wall resulting in ventricular dysfunction and congestive heart failure (CHF) [1]. However, results emerging from subsequent studies highlight an association between atrial fibrosis and CHF, in addition to it being implicated in valvular defects, hypertension and aging [2]. The association of atrial fibrosis with atrial fibrillation (AF) has been the subject of intense recent investigation since AF is the most commonly acquired arrhythmia in adults and substantially increases the risk of

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stroke and premature death in humans [2]. Rhythm and rate control drugs as well as surgical interventions are currently being used as therapies for AF [3]. Drugs targeting signaling pathways associated with atrial fibrosis and AF are also the focus of recent research based on their therapeutic potential in accessing downstream and potentially more specific targets for fibrosis. As a result, angiotensin II receptor blockers (ARBs), angiotensin converting enzyme inhibitors (ACE-Is), statins, anti-inflammatory and other (non anti-arrhythmic) agents have received attention as potential therapeutic interventions for AF and fibrosis [4,5]. There is now growing evidence that mineralocorticoid receptor (MR) activation is an important contributing factor to the development of atrial fibrosis and AF [6,7]. Spironolactone, a MR antagonist, is effective in reducing atrial fibrosis and associated AF in animal models and as a result is gaining increased attention as a potential therapy for human AF [6,8–10]; although the precise molecular mechanisms underlying its effects remain unclear. This review will discuss and highlight developments in the relationship between atrial fibrosis and AF, the role of spironolactone as a therapy for AF as well as distinct and common pathways regulating atrial and ventricular fibrosis (Figure 1). Since excess ECM deposition is a hallmark of fibrosis, this review will also discuss the importance of both qualitative and quantitative changes in relevant ECM proteins and its regulatory proteins within the context of atrial fibrosis (Figure 1).

Atrial Fibrosis and Atrial Fibrillation: A cause and effect relationship?

Atrial fibrosis and AF have been shown to occur concurrently but it is unclear whether there is a direct cause and effect relationship or whether these events occur as a consequence of independent pathologic changes in the heart [11]. A number of studies have been performed to establish whether fibrosis always accompanies AF. Clinically, histological analyses of atrial biopsy samples from patients with lone AF revealed that 25% of these patients exhibited no hallmarks of atrial fibrosis [12]. Similarly, in a goat model of AF, it was demonstrated that goats developed AF without significant effects on fibrosis [13]. However, other animal models of chronic atrial fibrosis, which utilized chronic rapid ventricular pacing or overexpression of transforming growth factor- β 1 (TGF- β 1) have an associated increase in atrial fibrosis with increased AF inducibility [8,14]. These data suggest that atrial fibrosis is sufficient but not necessary for induction of AF.

AF is also capable of enhancing atrial fibrosis. In human lone AF, it has been shown that long-term assessment of patients diagnosed with AF, which had normal sized atria upon diagnosis, does lead to structural remodeling of the atria causing atrial enlargement and dilatation over a subsequent period of 20 months [15]. Two studies, one which induced chronic atrial dilation in dogs through mitral regurgitation and another which induced CHF in dogs via rapid ventricular pacing, both showed an association between AF inducibility and atrial dilation/fibrosis [16,17]. These studies highlight a role for chronic atrial dilation in structural remodeling of the atria and demonstrate that it is a cause of both AF and atrial fibrosis. Furthermore, these studies suggest that atrial fibrosis acts as both a trigger and a by-product of AF, potentially through a mechanism affecting signaling pathways associated with atrial dilation. As a result, it is possible that the cyclical relationship between AF and atrial fibrosis may lead to a worsening heart condition with increased episodes of AF.

AF development stems from a combination of electrical, structural and contractile remodeling of the heart

Electrical Remodeling—The electrical changes in the atria are mainly due to a marked reduction in the L-type Ca^{2+} current with shortening of action potential duration, which results in shortening of the effective refractory period. This mechanism is involved in the occurrence and maintenance of atrial fibrosis [18]. Studies performed in a goat model of AF, demonstrated that goats exhibited a significantly reduced atrial effective refractory period, which caused a significant increase in the duration of AF [19]. This shortened effective refractory period had

a significant effect on the ability of cardiomyocytes to become reactivated during contraction, which in turn increased the atria's susceptibility to AF. It has been proposed by a number of groups that these electrical changes are associated with an early molecular loss or mislocalization of gap junction proteins (connexin 40 and 43) from the intercalated disc, suggesting that early gap junction remodeling could underlie the conduction abnormalities observed in AF [20,21]. However, a definitive association between connexin 43 and AF has yet to be clearly established, due to conflicting data in both clinical and animal studies [22]. Ionic remodeling is also a consequence of the electrical changes in CHF-related AF, but it can also occur as a secondary consequence to the structural changes associated with AF [23]. Linkage analysis studies have identified putative mutations in potassium ion channels in patients with AF; however, it remains to be determined whether ion channel remodeling can directly lead to the development of AF and associated atrial fibrosis in mice and humans [24].

Contractile Remodeling—Contractile remodeling is evidenced by a decrease in atrial contractility which can lead to dilation of the atria in fibrillating hearts [25]. Recent studies have implicated inositol-1,4,5-trisphosphate-receptor (IP₃R)-mediated Ca²⁺ release as playing an important role in the modulation of excitation-contraction coupling in atrial tissue and in the generation of AF. Studies using IP₃R2 deficient mice revealed that loss of IP₃R2 in atrial cells abolished the positive inotropic effect of neurohumoral stimulation with endothelin-1 (ET-1) and protected atrial cells from its arrhythmogenic effects, suggesting that calcium release channels could also contribute to the development of AF [26]. This idea is further supported by the importance of the reduction in L-type Ca²⁺ current, which is integral to excitation-contraction coupling in chronic AF [18].

Structural Remodeling—Structural remodeling within atria is evidenced by interstitial fibrosis and atrial dilatation. This increased space between cardiomyocytes, which is likely due to the loss of cells and fibrotic replacement and expansion of the ECM may also cause conduction delays between cardiomyocytes and allow for alternate pathways of conduction. These changes are electrically apparent as ectopic foci and anisotropic conduction (directional variations), which create non-uniform wave fronts that allow for abnormal re-entrant arrhythmias [27]. The effects of structural atrial remodeling on the development of AF were most clearly demonstrated in a cardiac myocyte specific transgenic mouse model over-expressing TGF-β1, which resulted in atrial fibrosis. The increase in atrial fibrosis significantly increased AF susceptibility within these transgenic mice [14], and suggested that enhancement of atrial fibrosis provides a sufficient substrate for AF.

Pharmacological Interventions for Fibrosis and AF

The Renin-Angiotensin-Aldosterone-System's Role in Atrial Fibrosis and AF—Aldosterone is a hormone involved in the renin-angiotensin-aldosterone system (RAAS), which affects multiple organs in the body including the kidneys and heart. The mechanism of aldosterone's action in the kidney has been widely studied, where it binds to the MR and increases sodium uptake and potassium secretion in the distal tubules. The effect of MR activation on cardiac fibrosis has been reviewed elsewhere [28]. Although the exact mechanisms and effects of aldosterone in the heart have yet to be clearly elucidated, previous studies using various mouse models have shown that increased aldosterone levels lead to the development of atrial fibrosis [29,30].

The subject of cardiac biosynthesis of aldosterone in humans is a controversial issue, specifically in regard to whether the aldosterone produced in the heart is produced at levels high enough to have an effect on cardiac pathology, and has been reviewed in detail [31]. Although aldosterone is primarily synthesized in the adrenal cortex, intracardiac production

of aldosterone has also been reported in rats [32]. It was found in a rat model of hypertension and cardiac injury mediated by angiotensin II (AngII) and the nitric oxide synthesis inhibitor, N^ω-nitro-L-arginine methyl ester (L-NAME), that removal of aldosterone via adrenalectomy or the selective aldosterone antagonist eplerenone, was sufficient to significantly reduce both myocardial and kidney damage [33]. These studies suggest that systemic aldosterone produced from the adrenal glands has a much greater contribution to the development of myocardial damage than locally produced aldosterone from the heart.

Mineralocorticoid Receptor Antagonists—MR antagonists have emerged as a potential therapy for fibrosis. One such drug is spironolactone, which was originally designed to block the effects of aldosterone at the renal tubular level [34]. The effect of spironolactone on morbidity and mortality in patients with severe heart failure was addressed in the Randomized Aldactone Evaluation Study (RALES) [35]. A subset of the 1663 enrolled patients who were in most cases being treated with a triple therapy of ACE-I, furosemide and digoxin, received 25 mg of spironolactone daily. The spironolactone group showed both a 30% reduction in risk of death and a 30% reduction in risk of hospitalization for cardiac complications, providing compelling evidence of spironolactone's efficacy in treating heart failure. In addition, analysis of pro-collagen levels in a subset of the RALES trial patients before and after six months of spironolactone treatment resulted in a decrease in serum levels of pro-collagen type III when compared to baseline levels [36]. Although this study highlights an association between spironolactone treatment and a reduction in serum collagen metabolites, the effects of spironolactone on myocardial fibrosis within atrial or ventricular tissue were not directly assessed. In a subsequent study on human subjects it was shown that spironolactone in combination with losartan, which is an ARB, can also improve heart rate variability and QT dispersion [37], suggesting effects of spironolactone on cardiac autonomic control and cardiac electrical stability, respectively, the latter of which is a predictor for ventricular arrhythmias.

The mechanisms underlying the positive effects of spironolactone in the heart are not well understood. In rats induced with heart failure through myocardial infarction, spironolactone treatment resulted in a significant reduction in the development of atrial and ventricular fibrosis, although treatment was unable to completely prevent the development of fibrosis [38]. Similarly in canines exhibiting CHF, treatment with spironolactone reduced fibrosis and the duration of episodes of AF but did not result in a complete preventative effect [8]. These results suggest that MR-independent pathways, which are not targeted by spironolactone, could also play a crucial role in the development of cardiac fibrosis. The effects of spironolactone on fibrosis appear to be independent of the blood pressure lowering effects of inhibiting aldosterone action. In rats with hyperaldosteronism, a dosage of spironolactone insufficient to cause a decrease in blood pressure caused a significant reduction in cardiac fibrosis [6]. Altogether these studies highlight the effectiveness of spironolactone in reducing cardiac fibrosis and AF.

Several studies have attempted to identify putative pathways affected by spironolactone. Myocardial damage induced in rats treated with aldosterone and salt could be reduced by spironolactone and the ARB, losartan, as evidenced by the reduced degree of myocardial fibrosis [9]. In the same study, spironolactone and losartan also decreased the expression of angiotensin II receptor type 1 (AT₁) in the heart. More recently, Nox2-containing NADPH oxidase, a downstream target of AngII that has been previously shown to cause interstitial fibrosis, was inhibited by treatment with spironolactone in mice [10]. These studies suggest an intersection between spironolactone and AngII signaling pathways in regards to myocardial fibrosis that remains to be elucidated. In addition, a recent study using small interfering RNA (siRNA) to knockdown MR in cardiac fibroblasts demonstrated that aldosterone can activate elastogenesis through MR-independent signaling pathways [39], highlighting the potential of a MR independent aspect to the mechanism of spironolactone's action.

Although spironolactone has received the most attention as an antifibrotic MR antagonist, eplerenone, a newer MR antagonist can also have similar effects in terms of reducing fibrosis in animal studies of myocardial fibrosis and improving patient prognosis in CHF [40,41]. Eplerenone is the first selective MR antagonist and has major advantages over the nonselective MR antagonist spironolactone since it cannot bind to progesterone and androgen receptors, which is associated with drug-induced gynecomastia, breast pain, and impotence [41]. Although human clinical studies involving the use of eplerenone (EPHESUS) appear promising [35,42], both the long-term effects on cardiac prognosis and beneficial effects on cardiac fibrosis in human hearts remain to be determined.

Angiotensin II Receptor Blockers—The potential of ARBs for treatment of fibrosis and AF is also being explored. The ARB, valsartan reduced the degree of myocardial fibrosis in hypertensive rats [43]. Similarly, candesartan, which is another ARB, reduced interstitial fibrosis and AF duration in a canine model of AF [44]. However, a recent clinical trial (GISSI-AF) studying the effects of valsartan within patients who had a history of atrial fibrillation associated with cardiovascular disease, diabetes, or left atrial enlargement, demonstrated no beneficial effect of valsartan on the recurrence of AF within these patients after one year [45]. The conflicting outcomes of ARBs in animal studies versus the GISSI-AF human clinical trial suggest that further research is required into exploiting the anti-fibrotic effects of ARBs in the treatment of atrial fibrosis and any related AF in humans. Some key studies to consider with future human clinical trials, which were not addressed with the current trial and that could highlight a beneficial outcome of ARBs, include studying the long-term effects of ARBs on atrial fibrosis and AF as well as the effects of ARBs on primary prevention of atrial fibrillation.

Signaling Pathways Underlying Atrial and Ventricular Fibrosis

The mechanisms underlying ventricular fibrosis have been relatively well characterized when compared to the mechanisms underlying atrial fibrosis, which have only recently been uncovered. Common triggers for both ventricular and atrial fibrosis are depicted in Figure 1 and include the activation of the renin-angiotensin-aldosterone-system (RAAS), inflammation, and oxidative stress [28,46]. The importance of these systems has been demonstrated through inhibition or ablation of key pathway components. Blockade of AngII and aldosterone results in antifibrotic responses [6,35]. Similarly, treatment of hypertensive rats with the anti-inflammatory drug, Tranilast, leads to prevention of myocardial fibrosis and collagen accumulation [5]. Oxidative stress blockade via cardiac myocyte specific overexpression of Nox2 in mice also resulted in a significant reduction in aldosterone-mediated fibrosis [10]. The peroxisome proliferator-activated receptor- γ (PPAR- γ) activator pioglitazone, which antagonizes the actions of AngII and possesses anti-inflammatory as well as antioxidant properties *in vitro*, has also been shown to successfully reduce CHF-associated atrial remodeling and promotion of AF in rabbits [47]. However, it is also now becoming apparent that chamber-specific (atria versus ventricle) regulation of fibrosis can occur when these pathways are activated to varying extents.

Fibrosis is not always present throughout the entire heart. In lone AF, patients can exclusively present with atrial fibrosis [12]. Recently a number of animal models have been generated which highlight this selectivity. Transgenic mice overexpressing angiotensin converting enzyme (ACE) under the control of an α -MHC promoter, display normal ventricles but hypertrophic and dilated atria with focal atrial fibrosis and AF [48]. Similarly, transgenic mice expressing a constitutively active TGF- β 1 mutant under the control of an α -MHC promoter display normal ventricles but fibrotic atria [49]. These studies imply that there are different underlying mechanisms involved in the development of atrial versus ventricular fibrosis, at least in terms of the AngII and TGF- β 1 signaling pathways. However, there is a level of complexity governing the differential development of fibrosis that has yet to be uncovered,

which is highlighted in the results of Paradis *et al.*, who report that overexpression of AT₁ receptors leads to fibrosis in both atria and ventricles [50]. This differential chamber-specific fibrotic response to ACE overexpression versus AT₁ receptor overexpression could be explained by the differential AT₁ receptor expression in the atria and ventricles [51]. One possibility could be that the number of AT₁ receptors available to bind circulating AngII within each chamber differ and as a result, intrinsically affects the occurrence of cardiac fibrosis.

Differences in morphological, secretory, and proliferative responses between atria and ventricles also exist. Studies have revealed that the atria display a more potent fibrotic response to various stimuli, highlighting the possibility that atrial and ventricular fibroblasts respond differentially to pathological stimuli [52]. The enhanced reactivity displayed by atrial fibroblasts could explain the preferential atrial fibrotic responses observed in various heart diseases. In a model of rat cardiac overload, AT₁ receptor levels were measured in the atria and ventricles before and after the development of a vascular shunt between the aorta and vena cava [51]. Endogenous AT₁ receptor levels were significantly higher in the atria compared to the ventricles, and enhancement of receptor levels following shunt development was significantly greater in the atria compared to the ventricles. These studies highlight significant chamber-specific differences in the endogenous regulation of AngII signaling mediators, which must be taken into account when treating these compartments independently. AngII signaling to aldosterone production has been previously defined as proceeding via activation of G(q/11)-proteins, to which the AT₁ receptor normally couples [53]. However, a recent study highlighted an alternative mechanism of AT₁ receptor-dependent aldosterone production mediated by the scaffolding protein beta-arrestin-1 (betaarr1) [54]. Thus, a secondary level of complexity could be that adapter/scaffolding proteins involved in mediating signaling pathways associated with fibrosis could also be differentially expressed in the atria versus ventricles. Further studies will be required to establish if other receptor-mediated signaling pathways associated with fibrosis are differentially expressed in the heart and whether this could contribute to the development of selective fibrosis in the atria as compared to the ventricles.

Regulation of Relevant ECM Proteins in Atrial Fibrosis

Collagen is the ECM protein most commonly examined in cases of atrial fibrosis, although other ECM proteins have also been shown to display altered expression levels. The ECM predominantly consists of fibrillar collagen, but also contains laminin, entactin, fibronectin, and fibrillin, which make up the basement membrane, and various proteoglycans [55]. Collagen types I and III help maintain the structural integrity of the myocardial tissue and help translate cardiomyocyte shortening into ventricular pumping. The basement membrane maintains cell-cell interaction and cell adhesion, and proteoglycans are considered to play an important role in adhesion and signaling.

Although collagen is used as the predominant marker of fibrosis, other ECM proteins may play a key role in defining the quality of the ECM. In dilated, ischaemic, and valvular cardiomyopathies, there is differential cardiac expression of a number of ECM proteins, including collagen, fibronectin and laminin [56], suggesting that different ECM protein levels may lead to different cardiac pathologies. The expression of other ECM proteins not directly assessed may also alter in response to fibrosis. In a model of canine heart failure, hearts were shown to display an upregulation of 8 collagen genes by greater than 10-fold. In addition, fibrillin-1 was upregulated 8-fold and fibronectin 5-fold [57]. These findings demonstrate a potential contribution from other basement membrane proteins in fibrosis. An increase in fibrillin-1 in the ECM of rat hearts following treatment with AngII has also been shown [58]. Similarly, examination of the atria in a porcine model of AF found a number of upregulated ECM proteins including fibrillin-1, fibronectin-1 and fibromodulin when compared to hearts in sinus rhythm [59]. These differences in protein expression are likely to lead to an altered

ratio of proteins in the ECM, which may contribute to the pathogenesis of atrial fibrosis. For example, fibrillin, in addition to its structural roles, has a functional role in signaling where it is involved in sequestering growth factors such as TGF- β and bone morphogenetic protein (BMP) [60]. An increase of fibrillin levels may cause a perturbation in additional signaling pathways leading to further pathology. However, there is currently a lack of detailed research on the role of non-collagenous ECM proteins in the context of atrial fibrosis. More research is necessary to establish whether the levels of these ECM proteins in combination cause distinct pathologies in the fibrotic heart.

Additional important contributing factors to ECM remodeling in the heart are matrix metalloproteinases (MMPs), which are a family of matrix proteases that are involved in the metabolism of ECM proteins, and endogenous tissue inhibitors of MMPs (TIMPs) [61]. A diverse set of stimuli, including increased oxygen-derived free radicals, can activate MMPs leading to an increase in fibrosis [61]. In parallel, downregulation of TIMPs can also result in an enhancement of fibrosis [61]. In a study by Mukherjee *et al.*, [62], chamber-specific alterations in collagen content, MMP and TIMPs were shown to exist in patients with CHF in the presence versus absence of AF suggesting a role for them in the control of structural remodeling associated with AF. In regard to this, a subsequent study by Moe *et al.*, [63] demonstrated that inhibition of MMPs leads to reduction in atrial fibrosis and AF inducibility in a canine model of CHF, a finding which also highlights MMPs as a potential therapeutic target for atrial fibrosis and AF.

Conclusions and Future Directions

There is strong evidence to demonstrate that atrial fibrosis plays a key role in the development and persistence of AF, highlighting the therapeutic potential of anti-fibrotic drugs as a means to successfully treat AF. Since AF and CHF often coexist in patients, these mechanisms can also be exploited in heart failure patients as well as other cardiac pathologies. The MR-mediated pathway is a clear therapeutic target for atrial fibrosis and AF, and may hold promise in its exploitation in patients with atrial fibrosis and AF. The MR antagonists already demonstrate promise in reducing the morbidity and mortality rate within heart failure patients [35,42]. The mechanisms underlying the effects of MR antagonists are not clear; however, spironolactone does reduce the up-regulation of ECM proteins and expression of certain AngII signaling targets. However, whether MR-mediated pathways can directly affect ECM metabolism and gene regulation and/or target AngII signaling pathways remains to be determined. Since certain mediators within the Ang II signaling axis are thought to preferentially mediate atrial as opposed to ventricular fibrosis, it remains to be determined whether MR-mediated pathways could also target AngII-mediated targets that are uniquely important for atrial fibrosis.

Some key experimental studies, which could drive the atrial fibrosis/AF field forward are outlined in Table 1. One of the limitations in studying atrial fibrosis and cardiac fibroblasts *in vivo* is the lack of a reliable and specific marker for cardiac fibroblasts, and thus the inability to target these particular cells *in vivo*. Studies aimed at identifying cardiac fibroblast specific genes or regulatory sequences within these genes to allow for targeted ablation and overexpression of genes to cardiac fibroblasts would significantly improve the ability to generate more appropriate mouse model systems to study atrial fibrosis (perhaps even ventricular fibrosis) and associated AF. Since the existing mouse models of atrial fibrosis were generated by cardiomyocyte specific overexpression of candidate genes, this suggests that genes expressed in cardiomyocytes can have bonafide effects on cardiac fibroblasts. Mouse models generated to express mutant proteins defective in their ability to be secreted would also be invaluable in understanding the mechanisms (paracrine versus intracrine) within cardiomyocytes and cardiac fibroblasts, which lead to atrial fibrosis. Equally important may be an understanding of the interactions between cardiomyocytes and fibroblasts within existing

and newly generated atrial fibrosis mouse models using 3D cell culture systems, since the absence of these interactions could play a pivotal role in the development of fibrosis [64]. A number of recent studies have highlighted new candidate genes, which have yet to be targeted to the heart via cardiac knockout or overexpression studies, that may be more specific downstream targets in the pathways associated with fibrosis. Genes of particular interest include diacylglycerol kinase zeta [65], plasminogen activator inhibitor type-1 [66], and connective tissue growth factor (CTGF) and its regulators [67,68]. Recent studies have also identified miR-133 and miR-30 as important mediators of collagen synthesis via post-transcriptional regulation of CTGF [67]. In terms of the outcome of MR antagonists in humans, more human research is necessary. Clinical trials, which assess the effects of MR antagonists in patients with atrial fibrosis and AF or those at risk of developing atrial fibrosis and AF alongside with histological analysis of atrial tissues will allow for a more complete determination of the drug's efficacy in this context. Direct comparisons between spironolactone and other antifibrotic drugs in terms of AF prevention will also shed light on both spironolactone's method of action as well as what kind of substrate is necessary for the maintenance of AF. Also, a direct assessment of the effects of MR-mediated pathways on human atrial (fibroblast and myocyte) cells, in terms of their electrophysiological properties and gene expression profiles following MR antagonist treatment, will also be pivotal in determining the direct effects of MR mediated pathways within the atria.

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References

1. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. *Circulation* 1991;83:1849–65. [PubMed: 1828192]
2. Mazzini MJ, Monahan KM. Pharmacotherapy for atrial arrhythmias: present and future. *Heart Rhythm* 2008;5:S26–31. [PubMed: 18456197]
3. Callans DJ. Apples and oranges: comparing antiarrhythmic drugs and catheter ablation for treatment of atrial fibrillation. *Circulation* 2008;118:2488–90. [PubMed: 19064689]
4. Bajpai A, Savelieva I, Camm AJ. Treatment of atrial fibrillation. *Br Med Bull* 2008;88:75–94. [PubMed: 19059992]
5. Kagitani S, Ueno H, Hirade S, Takahashi T, Takata M, Inoue H. Tranilast attenuates myocardial fibrosis in association with suppression of monocyte/macrophage infiltration in DOCA/salt hypertensive rats. *J Hypertens* 2004;22:1007–15. [PubMed: 15097242]
6. Brilla CG, Matsubara LS, Weber KT. Anti-aldosterone treatment and the prevention of myocardial fibrosis in primary and secondary hyperaldosteronism. *J Mol Cell Cardiol* 1993;25:563–75. [PubMed: 8377216]
7. Brilla CG, Pick R, Tan LB, Janicki JS, Weber KT. Remodeling of the rat right and left ventricles in experimental hypertension. *Circ Res* 1990;67:1355–64. [PubMed: 1700933]
8. Yang SS, Han W, Zhou HY, Dong G, Wang BC, Huo H, et al. Effects of spironolactone on electrical and structural remodeling of atrium in congestive heart failure dogs. *Chin Med J (Engl)* 2008;121:38–42. [PubMed: 18208664]
9. Robert V, Heymes C, Silvestre JS, Sabri A, Swynghedauw B, Delcayre C. Angiotensin AT1 receptor subtype as a cardiac target of aldosterone: role in aldosterone-salt-induced fibrosis. *Hypertension* 1999;33:981–6. [PubMed: 10205234]
10. Johar S, Cave AC, Narayanananicker A, Grieve DJ, Shah AM. Aldosterone mediates angiotensin II-induced interstitial cardiac fibrosis via a Nox2-containing NADPH oxidase. *FASEB J* 2006;20:1546–8. [PubMed: 16720735]

11. Thiedemann KU, Ferrans VJ. Left atrial ultrastructure in mitral valvular disease. *Am J Pathol* 1977;89:575–604. [PubMed: 145805]
12. Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo MA, Maseri A. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. *Circulation* 1997;96:1180–4. [PubMed: 9286947]
13. Remes J, van Brakel TJ, Bolotin G, Garber C, de Jong MM, van der Veen FH, et al. Persistent atrial fibrillation in a goat model of chronic left atrial overload. *J Thorac Cardiovasc Surg* 2008;136:1005–11. [PubMed: 18954643]
14. Verheule S, Sato T, Everett Tt, Engle SK, Otten D, Rubart-von der Lohe M, et al. Increased vulnerability to atrial fibrillation in transgenic mice with selective atrial fibrosis caused by overexpression of TGF-beta1. *Circ Res* 2004;94:1458–65. [PubMed: 15117823]
15. Sanfilippo AJ, Abascal VM, Sheehan M, Oertel LB, Harrigan P, Hughes RA, et al. Atrial enlargement as a consequence of atrial fibrillation. A prospective echocardiographic study. *Circulation* 1990;82:792–7. [PubMed: 2144217]
16. Verheule S, Wilson E, Everett Tt, Shanbhag S, Golden C, Olgin J. Alterations in atrial electrophysiology and tissue structure in a canine model of chronic atrial dilatation due to mitral regurgitation. *Circulation* 2003;107:2615–22. [PubMed: 12732604]
17. Knackstedt C, Gramley F, Schimpf T, Mischke K, Zarse M, Plisene J, et al. Association of echocardiographic atrial size and atrial fibrosis in a sequential model of congestive heart failure and atrial fibrillation. *Cardiovasc Pathol* 2008;17:318–24. [PubMed: 18402824]
18. Van Wagoner DR. Electrophysiological remodeling in human atrial fibrillation. *Pacing Clin Electrophysiol* 2003;26:1572–5. [PubMed: 12914605]
19. Wijffels MC, Kirchhof CJ, Dorland R, Allesie MA. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. *Circulation* 1995;92:1954–68. [PubMed: 7671380]
20. Dhein S. Role of connexins in atrial fibrillation. *Adv Cardiol* 2006;42:161–74. [PubMed: 16646590]
21. Luo MH, Li YS, Yang KP. Fibrosis of collagen I and remodeling of connexin 43 in atrial myocardium of patients with atrial fibrillation. *Cardiology* 2007;107:248–53. [PubMed: 16953110]
22. Dhein S, Polontchouk L, Salameh A, Haefliger JA. Pharmacological modulation and differential regulation of the cardiac gap junction proteins connexin 43 and connexin 40. *Biol Cell* 2002;94:409–22. [PubMed: 12566216]
23. Cha TJ, Ehrlich JR, Zhang L, Shi YF, Tardif JC, Leung TK, et al. Dissociation between ionic remodeling and ability to sustain atrial fibrillation during recovery from experimental congestive heart failure. *Circulation* 2004;109:412–8. [PubMed: 14718405]
24. Tsai CT, Lai LP, Hwang JJ, Lin JL, Chiang FT. Molecular genetics of atrial fibrillation. *J Am Coll Cardiol* 2008;52:241–50. [PubMed: 18634977]
25. Schotten U, de Haan S, Neuberger HR, Eijssbouts S, Blaauw Y, Tieleman R, et al. Loss of atrial contractility is primary cause of atrial dilatation during first days of atrial fibrillation. *Am J Physiol Heart Circ Physiol* 2004;287:H2324–31. [PubMed: 15256374]
26. Li X, Zima AV, Sheikh F, Blatter LA, Chen J. Endothelin-1-induced arrhythmogenic Ca²⁺ signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate(IP3)-receptor type 2-deficient mice. *Circ Res* 2005;96:1274–81. [PubMed: 15933266]
27. Eckstein J, Verheule S, de Groot NM, Allesie M, Schotten U. Mechanisms of perpetuation of atrial fibrillation in chronically dilated atria. *Prog Biophys Mol Biol* 2008;97:435–51. [PubMed: 18378284]
28. Young MJ. Mechanisms of mineralocorticoid receptor-mediated cardiac fibrosis and vascular inflammation. *Curr Opin Nephrol Hypertens* 2008;17:174–80. [PubMed: 18277151]
29. Brilla CG, Weber KT. Mineralocorticoid excess, dietary sodium, and myocardial fibrosis. *J Lab Clin Med* 1992;120:893–901. [PubMed: 1453111]
30. Sun Y, Ramirez FJ, Weber KT. Fibrosis of atria and great vessels in response to angiotensin II or aldosterone infusion. *Cardiovasc Res* 1997;35:138–47. [PubMed: 9302358]
31. Schmidt BM, Schmieder RE. Aldosterone-induced cardiac damage: focus on blood pressure independent effects. *Am J Hypertens* 2003;16:80–6. [PubMed: 12517689]
32. Silvestre JS, Robert V, Heymes C, Aupetit-Faisant B, Mouas C, Moalic JM, et al. Myocardial production of aldosterone and corticosterone in the rat. Physiological regulation *J Biol Chem* 1998;273:4883–91.

33. Rocha R, Stier CT Jr, Kifor I, Ochoa-Maya MR, Rennke HG, Williams GH, et al. Aldosterone: a mediator of myocardial necrosis and renal arteriopathy. *Endocrinology* 2000;141:3871–8. [PubMed: 11014244]
34. Hood WG Jr, Hill R Jr, Pittman JA Jr, Farmer TA Jr. Studies on the metabolic effects of spironolactone in man. *Ann N Y Acad Sci* 1960;88:864–80. [PubMed: 13715691]
35. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* 1999;341:709–17. [PubMed: 10471456]
36. Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES). Rales Investigators. *Circulation* 2000;102:2700–6. [PubMed: 11094035]
37. Shehab A, Elnour AA, Struthers AD. A randomised, controlled, double-blind, cross-over pilot study assessing the effects of spironolactone, losartan and their combination on heart rate variability and QT dispersion in patients with chronic heart failure. *Cardiovasc J Afr* 2008;19:292–6. [PubMed: 19104723]
38. Milliez P, Deangelis N, Rucker-Martin C, Leenhardt A, Vicaute E, Robidel E, et al. Spironolactone reduces fibrosis of dilated atria during heart failure in rats with myocardial infarction. *Eur Heart J* 2005;26:2193–9. [PubMed: 16141258]
39. Bunda S, Wang Y, Mitts TF, Liu P, Arab S, Arabkhari M, et al. Aldosterone stimulates elastogenesis in cardiac fibroblasts via mineralocorticoid receptor-independent action involving the consecutive activation of Gα13, c-Src, the insulin-like growth factor-I receptor, and phosphatidylinositol 3-kinase/Akt. *J Biol Chem* 2009;284:16633–47. [PubMed: 19372600]
40. Nishioka T, Suzuki M, Onishi K, Takakura N, Inada H, Yoshida T, et al. Eplerenone attenuates myocardial fibrosis in the angiotensin II-induced hypertensive mouse: involvement of tenascin-C induced by aldosterone-mediated inflammation. *J Cardiovasc Pharmacol* 2007;49:261–8. [PubMed: 17513943]
41. Barnes BJ, Howard PA. Eplerenone: a selective aldosterone receptor antagonist for patients with heart failure. *Ann Pharmacother* 2005;39:68–76. [PubMed: 15590870]
42. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* 2003;348:1309–21. [PubMed: 12668699]
43. Akashiba A, Ono H, Ono Y, Ishimitsu T, Matsuoka H. Valsartan improves l-NAME-exacerbated cardiac fibrosis with TGF-beta inhibition and apoptosis induction in spontaneously hypertensive rats. *J Cardiol* 2008;52:239–46. [PubMed: 19027602]
44. Kumagai K, Nakashima H, Urata H, Gondo N, Arakawa K, Saku K. Effects of angiotensin II type 1 receptor antagonist on electrical and structural remodeling in atrial fibrillation. *J Am Coll Cardiol* 2003;41:2197–204. [PubMed: 12821247]
45. Disertori M, Latini R, Barlera S, Franzosi MG, Staszewsky L, Maggioni AP, et al. Valsartan for prevention of recurrent atrial fibrillation. *N Engl J Med* 2009;360:1606–17. [PubMed: 19369667]
46. Zhao W, Zhao T, Chen Y, Ahokas RA, Sun Y. Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor-beta1 in hypertensive rats. *Mol Cell Biochem* 2008;317:43–50. [PubMed: 18581202]
47. Shimano M, Tsuji Y, Inden Y, Kitamura K, Uchikawa T, Harata S, et al. Pioglitazone, a peroxisome proliferator-activated receptor-gamma activator, attenuates atrial fibrosis and atrial fibrillation promotion in rabbits with congestive heart failure. *Heart Rhythm* 2008;5:451–9. [PubMed: 18313605]
48. Xiao HD, Fuchs S, Campbell DJ, Lewis W, Dudley SC Jr, Kasi VS, et al. Mice with cardiac-restricted angiotensin-converting enzyme (ACE) have atrial enlargement, cardiac arrhythmia, and sudden death. *Am J Pathol* 2004;165:1019–32. [PubMed: 15331425]
49. Nakajima H, Nakajima HO, Salcher O, Dittie AS, Dembowsky K, Jing S, et al. Atrial but not ventricular fibrosis in mice expressing a mutant transforming growth factor-beta(1) transgene in the heart. *Circ Res* 2000;86:571–9. [PubMed: 10720419]

50. Paradis P, Dali-Youcef N, Paradis FW, Thibault G, Nemer M. Overexpression of angiotensin II type I receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. *Proc Natl Acad Sci U S A* 2000;97:931–6. [PubMed: 10639182]
51. Bauer P, Regitz-Zagrosek V, Kallisch H, Linz W, Schoelkens B, Hildebrandt AG, et al. Myocardial angiotensin receptor type 1 gene expression in a rat model of cardiac volume overload. *Basic Res Cardiol* 1997;92:139–46. [PubMed: 9226099]
52. Burstein B, Libby E, Calderone A, Nattel S. Differential behaviors of atrial versus ventricular fibroblasts: a potential role for platelet-derived growth factor in atrial-ventricular remodeling differences. *Circulation* 2008;117:1630–41. [PubMed: 18347210]
53. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev* 2000;52:415–72. [PubMed: 10977869]
54. Lymperopoulos A, Rengo G, Zincarelli C, Kim J, Soltys S, Koch WJ. An adrenal beta-arrestin 1-mediated signaling pathway underlies angiotensin II-induced aldosterone production in vitro and in vivo. *Proc Natl Acad Sci U S A* 2009;106:5825–30. [PubMed: 19289825]
55. Kassiri Z, Khokha R. Myocardial extra-cellular matrix and its regulation by metalloproteinases and their inhibitors. *Thromb Haemost* 2005;93:212–9. [PubMed: 15711735]
56. Herpel E, Pritsch M, Koch A, Dengler TJ, Schirmacher P, Schnabel PA. Interstitial fibrosis in the heart: differences in extracellular matrix proteins and matrix metalloproteinases in end-stage dilated, ischaemic and valvular cardiomyopathy. *Histopathology* 2006;48:736–47. [PubMed: 16681691]
57. Cardin S, Libby E, Pelletier P, Le Bouter S, Shiroshita-Takeshita A, Le Meur N, et al. Contrasting gene expression profiles in two canine models of atrial fibrillation. *Circ Res* 2007;100:425–33. [PubMed: 17234964]
58. Bouzeghrane F, Reinhardt DP, Reudelhuber TL, Thibault G. Enhanced expression of fibrillin-1, a constituent of the myocardial extracellular matrix in fibrosis. *Am J Physiol Heart Circ Physiol* 2005;289:H982–91. [PubMed: 15849235]
59. Lin CS, Lai LP, Lin JL, Sun YL, Hsu CW, Chen CL, et al. Increased expression of extracellular matrix proteins in rapid atrial pacing-induced atrial fibrillation. *Heart Rhythm* 2007;4:938–49. [PubMed: 17599682]
60. Ramirez F, Rifkin DB. Extracellular microfibrils: contextual platforms for TGFbeta and BMP signaling. *Curr Opin Cell Biol.* 2009
61. Spinale FG. Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function. *Physiol Rev* 2007;87:1285–342. [PubMed: 17928585]
62. Mukherjee R, Herron AR, Lowry AS, Stroud RE, Stroud MR, Wharton JM, et al. Selective induction of matrix metalloproteinases and tissue inhibitor of metalloproteinases in atrial and ventricular myocardium in patients with atrial fibrillation. *Am J Cardiol* 2006;97:532–7. [PubMed: 16461051]
63. Moe GW, Laurent G, Doumanovskaia L, Konig A, Hu X, Dorian P. Matrix metalloproteinase inhibition attenuates atrial remodeling and vulnerability to atrial fibrillation in a canine model of heart failure. *J Card Fail* 2008;14:768–76. [PubMed: 18995182]
64. Baudino TA, McFadden A, Fix C, Hastings J, Price R, Borg TK. Cell patterning: interaction of cardiac myocytes and fibroblasts in three-dimensional culture. *Microsc Microanal* 2008;14:117–25. [PubMed: 18312716]
65. Hirose M, Takeishi Y, Niizeki T, Shimojo H, Nakada T, Kubota I, et al. Diacylglycerol kinase zeta inhibits G(alpha)q-induced atrial remodeling in transgenic mice. *Heart Rhythm* 2009;6:78–84. [PubMed: 19121805]
66. Zaman AK, French CJ, Schneider DJ, Sobel BE. A profibrotic effect of plasminogen activator inhibitor type-1 (PAI-1) in the heart. *Exp Biol Med (Maywood)* 2009;234:246–54. [PubMed: 19144865]
67. Duisters RF, Tijssen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, et al. miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. *Circ Res* 2009;104:170–8. 6p following 8. [PubMed: 19096030]
68. Recchia AG, Filice E, Pellegrino D, Dobrina A, Cerra MC, Maggolini M. Endothelin-1 induces connective tissue growth factor expression in cardiomyocytes. *J Mol Cell Cardiol* 2009;46:352–9. [PubMed: 19111553]

Figure 1. A schematic representation outlining the complex interplay regulating atrial fibrosis and atrial fibrillation development

(Right) Schemata of the physiological pathways (oxidative stress, inflammation, and RAAS) thought to be involved in the development of atrial and ventricular fibrosis. **(Left)** Red arrows delineate the major contributing factors leading to the cyclical relationship between atrial fibrosis and AF. AF development stems from a combination of electrical, structural and contractile remodeling in the heart, which are highlighted in blue. Clinical outcomes of atrial fibrosis, ventricular fibrosis and AF are outlined in green. Potential therapeutic approaches are outlined in orange and include treatment with MR antagonists like spironolactone, angiotensin type II receptor blockers and the specific targeting of miRNAs involved in the regulation of fibrosis/collagen synthesis.

Key Studies Addressing Atrial Fibrosis and AF

Table 1

Model	Goal	Experimental Studies
Mouse	To generate cardiac fibroblast specific conditional mouse models for the study of atrial fibrosis and AF	Identification of cardiac fibroblast specific genes and regulatory regions within cardiac fibroblast specific genes which are necessary to target cardiac fibroblast specific expression
Mouse	To determine whether paracrine and/or intracrine mechanisms in cardiomyocytes and fibroblasts lead to atrial fibrosis and AF	Cardiomyocyte specific expression of mutant proteins within mice, which result in loss in their ability to become secreted
Mouse	To determine the role of cardiomyocyte-fibroblast cell interactions in the development of atrial fibrosis and AF	Generate and analyze 3D culture (myocyte-fibroblast) systems from existing and newly generated mouse models of atrial fibrosis and AF
Mouse	To determine the role of more specific and downstream targets, associated with atrial fibrosis and AF, as a means to identify new mechanisms	Generate cardiac-specific mouse models with targeted deletion or overexpression of new candidate genes, such as diacylglycerol kinase zeta, plasminogen activator inhibitor type-1, connective tissue growth factor, miR-133, miR-30
Human	To determine the effects of MR antagonists in human atrial fibrosis and AF	Perform randomized human clinical trials on MR antagonists with patients that have existing atrial fibrosis and AF as well as those at risk for developing atrial fibrosis and AF.
Human	To determine the direct role of MR-mediated pathways in human atrial cells (cardiomyocytes and fibroblasts)	Perform electrophysiological and gene expression studies in human atrial cells following MR antagonist treatment