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Gene therapy for atrial fibrillation in heart failure

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Introduction

Atrial fibrillation (AF) is the most common arrhythmia and a major cause of morbidity and mortality in an aging population. Unfortunately, current treatments for AF are suboptimal, in large part because the molecular mechanisms underlying AF are not well understood. Recent advances in our understanding of the AF disease state have led to the pre-clinical development of gene-based therapies that are targeted to key molecular mechanisms involved in the genesis and maintenance of AF.

Need for new, mechanism-targeted therapies in persistent AF and AF resulting from congestive heart failure

Atrial fibrillation (AF) is the most common heart rhythm disorder and is a major cause of stroke and heart failure (HF). Unfortunately, current therapies for AF, including drugs and ablation are suboptimal in the setting of persistent AF and structural heart disease (e.g. HF). Since a majority of AF triggers and drivers originate in the pulmonary veins (PVs) and posterior left atrium (PLA), catheter ablation to electrically isolate the PVs/PLA has recently emerged as a viable therapy for AF. Nonetheless, high ablation success rates have only been achieved in selected patients. Even with extensive, linear ablation in the atrium – which can lead to a decrease in atrial contractility and an increased incidence of complications – ablation success in the setting of structural heart disease does not appear to exceed 50–60%. The limited efficacy of current treatment options has led to a major research effort to better understand the mechanisms underlying this arrhythmia. A better understanding of the molecular mechanisms underlying electrophysiological remodeling in the atrium has also led to recent efforts to selectively target some of these mechanisms by using a biological i.e. gene-based approach.

The development of AF is heralded by electrophysiological and structural alterations which serve to maintain, promote and propagate AF¹. Electrophysiological (electrical) remodeling in the atrium is typically characterized by refractory period shortening, as well as changes in conduction. Of the structural changes that occur in AF, fibrosis is considered especially important in the generation of the substrate leading to AF, especially in the setting of HF, with fibrosis likely contributing to reentry by causing inhomogeneous conduction in the

atrium². In this review, we will discuss recent attempts to target both electrical and structural substrate in the atria of large animals with HF.

Gene therapy targeting electrical remodeling in the atrium in congestive heart failure

Of the gene-based approaches examined thus far in pre-clinical studies, most have targeted AF in a porcine model where rapid atrial pacing is used to induce AF. This model not only leads to rapid atrial pacing induced electrical remodeling in the atrium, but also leads to some decrease in left ventricular systolic function (HF) due to rapid ventricular rates during rapid atrial pacing. The majority of these studies have targeted ion channels or gap junctions that contribute to electrical remodeling in the atrium (i.e. ERP shortening or conduction slowing). Amit et al and Soucek et al showed that refractory period shortening in AF can be prevented in this model by over-expression of dominant negative mutants of the I_{Kr} channel, with a resulting decrease in AF^{2,3}. Since conduction changes also contribute to AF, Igarashi et al and Bikou et al showed that gene transfer of Connexin 40 and/or 43 led to improved conduction and reduced AF relative to controls in the same rapid atrial pacing model of AF².

Very recently, Lugenbiel et al⁴ showed in the same porcine model AF with reduced ventricular function (HF) that downregulation of atrial repolarizing TREK-1 ($K_{2p2.1}$) K^+ channels may be contributing to electrical remodeling during AF with HF. Although rapid atrial pacing typically leads to refractory period shortening, the onset of HF due to rapid ventricular rates can eventually lead to prolongation of the atrial effective refractory period. The investigators showed that overexpression of TREK-1 gene in the atrium leads to shortening (normalization) of atrial effective refractory periods in pigs with HF, with a resulting decrease in AF. Surprisingly, restoration of sinus rhythm also led to a decrease in fibrosis in the right atrium. This was thought to be due to a combined effect of rhythm control, reduced ventricular rates, and improved cardiac function in TREK-1 compared to sham-treated AF pigs.

In addition to targeting ion channels and gap junctions, some reports suggest that targeting of autonomic signalling in the atrium can also have beneficial effects on AF substrate. Genetic upregulation of $G\alpha_i$ protein or genetic suppression of $G\alpha_s$ protein in the porcine AV node provides rate control in AF³. In related studies our group has targeted autonomic signaling in the atrial myocardium instead of the AV node, and shown that vagal induced AF in normal dogs can be successfully prevented by atrial injection of plasmid expressing C-terminal $G\alpha_i$ and/or $G\alpha_o$.⁵

Gene therapy targeting structural remodeling in the atrium in congestive heart failure

More recently, there have been efforts to use a gene-based approach to target structural substrate for AF in the setting of HF. The structural changes that typically occur in the fibrillating atrium including fibrosis, hypertrophy, necrotic and apoptotic cell loss, and dilation. Using the above-mentioned porcine model, Trappe et al have shown that

knockdown of caspase 3 by atrial Ad-siRNA-Cas3 gene transfer suppresses or delays the onset of persistent AF by reduction in apoptosis and prevention of intra-atrial conduction delay².

Of the morphological changes that occur in the fibrillating atrium, fibrosis is considered especially important in the creation of AF substrate – both in the absence and presence of HF. Patients with AF display increased atrial fibrous tissue content, along with increased expression of collagen I and III, up-regulation of MMP-2 protein, and down-regulation of the tissue inhibitor of metalloproteinase, TIMP-1.

Although the molecular mechanisms underlying the development of fibrosis are complex, several studies suggest that TGF- β signaling is an important contributor to cardiac fibrosis, as evidenced by over-expression and knockout models. TGF- β signaling facilitates phenocconversion of cardiac fibroblasts to activated myofibroblasts, thereby stimulating fibroblast production of collagen, fibronectin, proteoglycans. TGF- β signaling also promotes apoptosis, which can indirectly lead to replacement fibrosis. Both SMAD2/3 mediated canonical TGF- β signaling and non-canonical signaling (ERK1/2, p38 MAP kinase) have been implicated in TGF- β induced fibrosis in the heart. TGF- β also activates ROS, with redox-sensitive signaling pathways mediating TGF- β -induced cardiac hypertrophy, fibrosis, and structural remodeling. Lately, TGF- β has been shown to be involved in creation of atrial fibrosis. Serum levels of TGF- β have been shown to be increased in AF patients undergoing cardioversion. Moreover, transgenic over-expression of TGF- β in the mouse causes selective atrial, but not ventricular fibrosis, which results in altered conduction characteristics and increased AF inducibility². Indeed, microarray analyses demonstrate upregulation of TGF- β in atria of patients with AF secondary to valvular heart disease².

In view of the above, TGF- β signaling in the atrium appears to be a viable therapeutic target in AF. We hypothesized that a targeted, gene-based strategy that selectively inhibits canonical TGF- β signaling in the atrium – and more specifically in the PLA - would be sufficient to attenuate formation of fibrotic substrate in AF in a canine model of ventricular tachypacing induced HF (that is known to cause AF substrate by leading to the development of marked interstitial fibrosis in the atria). Therefore, in dogs undergoing ventricular tachypacing, we performed targeted injection in the PLA a plasmid expressing a kinase-deficient, dominant negative TGF- β type II receptor (TGF- β -RII DN) under the control of a long acting human polyubiquitin C (UBC) promoter. Our results illustrate that: a) gene-based targeting of TGF- β signaling in the canine atrium is feasible, with a plasmid expressing TGF- β -RII-DN, b) inhibition of TGF- β signaling in the PLA by TGF- β -RII-DN resulted in a significant decrease in HF-induced atrial fibrosis (see figure 1), leading to a significant decrease in the conduction heterogeneity that characterizes fibrosis-induced AF substrate, c) TGF- β -RII-DN expression results in flattening of the restitution slope in the PLA and d) the improvement in conduction and restitution characteristics translates into a significant decrease in the duration of inducible AF. In addition, our results demonstrate that a decrease in atrial fibrosis by TGF- β inhibition is accompanied by a significant change in the complexity and organizational characteristics of induced AF. To our knowledge, this is the first report of a targeted gene-therapy approach to decrease atrial fibrosis in a clinically relevant large animal model of AF, with a view towards modifying AF substrate. TGF- β -

RII-DN not only decreased atrial fibrosis in that study, but appeared to do so by affecting both canonical (pSMAD2/3 mediated) and non-canonical (i.e. pERK1/2 mediated) TGF- β signaling. Since TGF- β -RII-DN was found to attenuate pERK1/2 not only in atrial fibroblasts but also in atrial myocytes, it is possible that at least some of the beneficial effects of TGF- β -DN-RII on atrial fibrosis may be mediated by paracrine cross-talk between myocytes and fibroblasts.

Future directions

Just as targeting key signaling pathways in the ventricle with a gene-based approach – such as upregulating SERCA2a to help normalize excitation-contraction coupling in the ventricular myocytes – has shown some promise in reversing the HF disease state, similar approaches that target signaling pathways integral to electrical and structural remodeling in AF are likely to allow beneficial remodeling in the fibrillating atrium. In addition to discovering new molecular targets for gene therapy, a key challenge for gene therapy in AF will be need to develop minimally invasive approaches to facilitate safe and effective gene delivery in the atrium. These delivery techniques would include not only the means to inject gene cargo in the atrium, but also – at least in the case of non-viral vectors – a means to facilitate intracellular gene delivery e.g. by a physical method such as electroporation.

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Figure 1. Effect of plasmid expressing a dominant-negative TGF- β type II receptor (pUBc-TGF β -DN-RII) on fibrosis. Panel I, Example of dense, interstitial fibrosis in posterior left atrium (PLA) from 2 dogs receiving pUBc-*LacZ*. Panel II, PLA from 2 dogs receiving pUBc-TGF β -DN-RII shows very little interstitial fibrosis. Fibrosis is stained blue. Panel III, % dense, interstitial fibrosis in pUBc-*LacZ* vs pUBc-TGF β -DN-RII injected PLA (*modified from, Kunamalla A et al. Constitutive Expression of a Dominant-Negative TGF-beta Type II*

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