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Rapid communication

Transcriptomic analysis reveals atrial *KCNE1* down-regulation following lung lobectomy

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ABSTRACT

Lone atrial fibrillation (AF) is associated with various ion channel gene sequence variants, notably the common S38G loss-of-function polymorphism in the *KCNE1* K^+ channel ancillary subunit gene. New-onset postoperative AF (POAF) generally occurs 48–72 h after major surgery, particularly following procedures within the chest, but its molecular bases remain poorly understood. To begin to address this gap in knowledge, we analyzed molecular changes in the left atrium (LA) in relation to simultaneous changes in hemodynamics, LA effective refractory period (ERP), and the capacity to sustain electrically-induced AF following left upper lung lobectomy in swine. Relative to control pigs (no previous surgery), 3 days after lobectomy higher values for mean pulmonary artery pressure (16 ± 1 vs 22 ± 2 mm Hg; $P = 0.045$) and pulmonary vascular resistance (273 ± 47 vs 481 ± 63 dyn s/cm^5; $P = 0.025$) were evident, whereas other hemodynamic variables were unchanged. LA ERP trended toward reduction in lobectomy animals (187 ± 16 vs 170 ± 20 ms, $P > 0.05$). None of the lobectomy pigs developed spontaneous POAF as assessed by telemetric ECG. However, all lobectomy pigs, but none of the controls, were able to sustain AF induced by a 10 s burst of rapid pacing for ≥30 s ($P = 0.0079$), independent of LA ERP; AF was sustained ≥60 s in 3/5 postoperative pigs versus 0/5 controls and correlated with a shorter ERP overall ($P = 0.023$). Transcriptomic analysis of LA tissue revealed 23 up-regulated and 10 down-regulated transcripts (≥1.5-fold, $P < 0.05$) in lobectomy pigs. Strikingly, of the latter, *KCNE1* down-regulation showed the statistically strongest link to surgery (2.0-fold, $P = 0.009$), recapitulated at the protein level with Western blotting ($P = 0.039$), suggesting *KCNE1* down-regulation as a possible common mechanistic factor in POAF and lone AF. Of the up-regulated transcripts, while *Teneurin-2* was the strongest linked (1.5-fold change, $P = 0.001$), *DSCR5* showed the highest induction (2.7-fold, $P = 0.02$); this and other hits will be targeted in future functional studies.

1. Introduction

Atrial fibrillation (AF) is a cardiac arrhythmia associated with rapid, irregular atrial activation. AF represents an enormous burden in terms of healthcare costs, with an estimated 2.3 million sufferers in the United States alone. While most cases of AF are linked to underlying structural defects in the heart, lone or idiopathic AF, i.e., that in the absence of structural heart disease, has been associated with variants in genes encoding ion channel subunits. For example, a rare variant in the gene encoding the KCNE1 ancillary (β) subunit, which together with the KCNQ1 pore-forming (α) subunit forms the cardiac I_{Ks} potassium channel complex, was recently found to associate with AF [1], while individuals with two G alleles at the KCNE1 S38G polymorphic site (38GG) were found in one study to have a >10-fold increased risk of lone AF compared to other genotypes [2].

Another common form of AF, new-onset postoperative AF (POAF), increases postoperative stays and in-hospital patient mortality [3]. Not surprisingly, the highest incidence, up to 65%, has been reported following cardiac surgery where the pericardium was opened and the atria directly manipulated. However, even following non-cardiac surgery in the chest such as lung resection, a POAF incidence of >30% has been reported in some cohorts, with most episodes typically occurring within the first 2–3 postoperative days [4]. As inflammation and activation of the sympathetic nervous system are considered particularly important factors in the etiology of POAF, it is thought to be mechanistically somewhat distinct from other forms of AF, but the

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molecular basis for POAF is still incompletely understood. To begin to address this, we developed a large animal lung resection model of susceptibility to POAF. Here we report findings from a global whole-transcript analysis of postoperative gene remodeling in left atrial (LA) tissue obtained 3 days after removal of the left upper lung lobe in swine. Of the 25,388 genes probed, 10 genes were down-regulated ≥1.5-fold in the postoperative pigs compared to controls. Strikingly, *KCNE1* exhibited the strongest statistical link to postoperative remodeling.

2. Methods

Young adult Sinclair swine were divided into 'control' ($n=4$) and 'postop' ($n=5$) groups, the latter undergoing thoracotomy, removal of the left upper lung lobe, and implant of a transmitter for telemetric ECG recording prior to study. As the first procedure in controls and on the third postoperative day for the postop group, animals were anesthetized and instrumented for measurement of baseline systemic and pulmonary hemodynamic variables. After ganglionic blockade to isolate the myocardium from autonomic nervous input and its potential effects on ERP and AF induction, as we previously described [5], left atrial (LA) effective refractory period (ERP) was measured from a 300 ms cycle length. Atrial fibrillation was then induced by rapid burst pacing (10 s) and the duration of sustained AF after cessation of pacing determined. Animals were then euthanized, tissue harvested, and total RNA and total protein isolated from tissue homogenates for whole-transcript genomics and Western blotting studies, respectively. Global whole-transcript analysis was performed using a *GeneAtlas* microarray system (Affymetrix) and Porcine Gene 1.1 ST Array Strips (Affymetrix), each array comprising 572,667 probes to probe 25,388 genes (median probe set per transcript of 25, hence 'whole-transcript' analysis). Gene expression changes between control and surgery groups were analyzed in an unbiased fashion, using a ≥1.5-fold change, $P<0.05$ significance (Student's *t*-test), and mean transcript signal intensity in either group of ≥5.0 as cutoffs. Detailed methods are available in the online Supplementary Methods section.

3. Results

In postop pigs, mean pulmonary artery pressure was increased relative to control (16 ± 1 vs 22 ± 2 mm Hg; $P=0.045$) as was pulmonary vascular resistance index (273 ± 47 vs 481 ± 63 dyn s/cm^5; $P=0.025$). There were no significant differences in other measured hemodynamic variables (Supplementary Table 1). LA ERP trended toward reduction in postoperative animals (from 187 ± 16 to 170 ± 20 ms, $P>0.05$).

Rapid burst pacing was sufficient to induce AF with characteristic hemodynamic effects (Fig. 1A). All the postop pigs, but none of the controls, sustained AF for ≥30 s following rapid burst pacing ($P=0.0079$), independent of LA ERP (Fig. 1B). AF ≥60 s occurred in 3/5 postoperative pigs versus 0/5 controls and correlated with a shorter LA ERP when values from control and postoperative pigs were pooled ($P=0.023$) (Fig. 1 C).

Whole-transcript microarray analysis of LA tissue revealed 33 genes with expression changes ≥1.5-fold in postoperative pigs (Table 1). Of the 10 transcripts down-regulated ≥1.5-fold, *KCNE1* showed the statistically strongest link to postoperative changes (2.01-fold reduced, $P=0.009$) (Figs. 1D, E), followed by NPPB (Natriuretic peptide B precursor) and PTX3 (Pentraxin 3), which showed more variability but the strongest down-regulation (4.08-fold, $P=0.03$). *KCNE1* transcript down-regulation was reflected at the protein level, with a 40% reduction in KCNE1 protein band density in LA tissue from 3-day postoperative pigs compared to controls ($n=4$–5, $P=0.039$) (Fig. 1F), while GAPDH protein expression was unchanged ($n=4$–5, $P=0.47$) (Fig. 1G), recapitulating the microarray data ($P=0.60$) (Fig. 1E). Left atrial transcripts for 23 genes were up-regulated ≥1.5-fold in postoperative pigs, the strongest-correlated being *TNM2* (Teneurin 2) with a signal-to-noise ratio (*F*) of 35.3; and the most up-regulated (2.65-fold) being *DSCR5* (Down syndrome critical region 5) (Table 1).

4. Discussion

To our knowledge this is the first study to examine global atrial tissue transcript remodeling in the acute postoperative period following open-chest surgery (cardiac or non-cardiac). The strong correlation of atrial *KCNE1* down-regulation to the postoperative state revealed here, measurable just 3 days after surgery, is of great interest given that multiple prior studies indicated a correlation between lone AF and the *KCNE1* 38G polymorphism (e.g., [2]), which reduces KCNQ1-KCNE1 (I_{Ks}) current density in vitro [6]. Whether *KCNE1* down-regulation in POAF is a compensatory response or a factor contributing to the substrate for POAF remains to be established, but our findings provide the first evidence of this common molecular factor in lone AF and POAF, and suggest the possibility that *KCNE1* polymorphisms might influence incidence of POAF in human subjects who have undergone thoracic surgery.

Lone AF-associated point mutations in KCNE1 are generally thought to increase I_{Ks} density and thereby shorten atrial myocyte action potentials [1]. In the present study of POAF, shortened LA ERP associated with the highest propensity for AF (Fig. 1C), yet atrial KCNE1 expression was reduced in the postoperative group. The loss-of-function effects of the KCNE1 38G allele were suggested, based on in silico modeling, to have the potential to predispose to early afterdepolarizations [6], and this is potentially occurring in the current POAF model. However, one of two further alternatives is considered more likely: either the reduced KCNE1 expression alters repolarization in such a way as to provide a substrate for reentry by increasing the dispersion of ERP, or the KCNE1 remodeling we observe is not contributing to POAF but is instead a compensatory response to shortening of the LA ERP by other mechanisms. Lastly, while it is important to recognize that there are profound differences in the molecular correlates of the K^+ currents in the atria of large animals versus mice, it is theoretically possible that reduced KCNE1 expression actually *increases* porcine atrial myocyte K^+ current by mechanisms independent of KCNQ1, as observed in the *Kcne1* null mouse, which exhibits AF in the absence of structural heart disease, and increased atrial I_K [7].

In addition to KCNE1, the results suggest a number of other possible contributors to POAF susceptibility, and additional work will now be directed toward understanding cause and effect relationships versus compensatory remodeling scenarios for these other transcripts (Table 1). Briefly, it is fascinating that *PTX3* and *NPPB* (and, likewise, NPR3) were down-regulated in the postoperative group, given the strong positive correlation of the protein products of these genes with myocardial damage and their widespread use as biomarkers; it is suggested that the decrease in *PTX3* reflects increased utilization (and therefore release) of inflammation competent cells. Among the other proteins whose transcripts were down-regulated, *FHL1C* (Four and a half LIM domain protein isoform C) reduction could increase atrial Kv1.5 activity [8], potentially shortening the LA ERP. Aminopeptidase O isoform 1 is a metalloproteinase putatively involved in the renin angiotensin pathway [9]; Nestin is implicated in myocardial regeneration [10]; podoplanin is implicated in sinoatrial node development [11]; Homer 2 inhibits RyR2 channel function, and therefore Homer 2 down-regulation could result in increased RyR2 activity, as was recently observed in chronic AF patients [12].

For the proteins encoded by the postoperatively up-regulated transcripts, as might be expected many are involved in inflammation such as those in the complement cascade, consistent with an inflammatory component to POAF [13]. Also of note, PIK3CG synthesizes