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Quantitative Morphological Analysis of Super-resolution Images Provides Validation of Novel Therapies to Prevent Atrial Fibrillation

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Atrial fibrillation (AF) is the most common form of irregular cardiac rhythm (arrhythmia), affecting ~3% of the US population. It is widely associated with vascular leak and inflammation and several risk factors are known to increase the incidence of new onset AF, including hypertension, diabetes, myocardial infarction, and cardiac surgery [1]. Despite its high prevalence and poor outcomes, the mechanism underlying AF is not well understood and current treatments are limited to managing this progressive disease, rather than arresting the underlying pathology.

AF patients have elevated levels of the inflammatory cytokine vascular endothelial growth factor (VEGF-A; 90-580 pg/ml), which promotes vascular leak and edema [2-4]. We investigated the structural underpinnings of AF using a novel approach we developed called indirect correlative light and electron microscopy (*iCLEM*). By exploiting structural landmarks identifiable via both light and electron microscopy, we were able to correlate transmission electron microscopy (TEM) –derived cardiac myocyte ultrastructure with molecular organization, assessed using sub-diffraction confocal imaging (sDCI) and stochastic optical reconstruction microscopy (STORM). Using this approach, we recently demonstrated that the vascular leak-inducing cytokine vascular endothelial growth factor (VEGF; 90-580 pg/ml – levels found in AF patients) induces acute remodeling (30-60 minutes) of sodium channel ($Na_V1.5$) –rich intercalated disk (ID) nanodomains, disrupting their ultrastructure and prompting translocation of $Na_V1.5$ from these sites [5]. This in turn disrupted atrial impulse propagation (high speed optical mapping) and promoted atrial arrhythmias (electrocardiography). Here, we tested the hypothesis that protecting the vascular endothelial barrier may prevent arrhythmias following an acute inflammatory insult by preserving cardiac ID nanodomains.

We used our murine model of AF induced by acute inflammatory insult (100 pg/ml VEGF for 60 minutes) to test the antiarrhythmic efficacy of protecting the vascular endothelial barrier. We identified two molecular targets for vascular barrier protection, connexin43 (Cx43) and pannexin-1 (Panx1) [6,7]. In vascular endothelial cells, the opening of connexin43 hemichannels and pannexin channels, providing electrical and chemical coupling between cells and extracellular space, has been implicated in cytokine-induced vascular leak. Median *in vivo* arrhythmia burden was higher in VEGF–treated mice relative to vehicle controls (7.5±11 vs. 0±6 s/hr). VEGF shifted $Na_V1.5$ signal to longer distances from Cx43 gap junctions (GJs), measured by a distance transformation-based spatial analysis of 3D confocal images of IDs. Further, VEGF decreased non-random attraction of $Na_V1.5$ to Cx43 GJs (0.43 vs. 0.58 in vehicle controls), where 0 indicates random distribution of $Na_V1.5$ in relation to Cx43 GJs and 1 indicates total superposition. Similar effects were observed with $Na_V1.5$ localized near mechanical junctions (MJs) composed of N-cad. Blocking connexin43 hemichannels (αCT11 peptide) decreased *in vivo* arrhythmia burden to 0 ± 6.07 s/hr. PxIL2P (a peptide inhibitor of Panx1 channels) treatment also decreased *in vivo* arrhythmia burden ((0 ± 6.09 s/hr with 1.6 μM PxIL2P). Concurrently, both peptide therapies preserved $Na_V1.5$ distance from GJs to control levels (0.50 with PxIL2P and 0.53 with αCT11) in these hearts.

Once again, similar effects were observed near N-cad MJs. Notably, similar antiarrhythmic efficacy was also achieved with clinically relevant small molecule inhibitors of Cx43 and Panx1.

In summary, these results highlight inflammation-induced vascular leak as a novel AF mechanism, and present novel therapies to prevent AF with robust mechanistic validation.

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