**Treat the Patient, Not Just the Cell!**

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Recently we have used reductionist and integrative approaches as pioneered by the seminal work of Sanguinetti and Keating to link human arrhythmias with gene mutations that alter the biophysical activity of channel proteins. But as we begin to seek new methodologies to treat heart disease, we are faced with a myriad of data that clearly simple cell-based assays or culture models cannot address on their own. Is it time to change course?

We all remember when in our ninth grade biology class, we first learned about the cell membrane. It was after all, critically important in that it held in the essential components of a cell allowing it to function. Many of us continue to be fascinated by the proteins (ion channels, transporters, and receptors) that traverse the membrane to synchronize cellular function at baseline and in response to physiological or pathological signaling. Over the past 2 decades, we have refined our approaches from whole animal physiology to highly quantitative biophysical, structural, and molecular technologies. These approaches, combined with refined human clinical phenotyping, have illustrated that gene variants in membrane proteins, particularly ion channels, are the source of potentially fatal forms of arrhythmia. Such an approach was pioneered by the work of Sanguinetti et al1 that linked long-QT syndrome with gene variants that altered the biophysical activity of voltage-gated potassium channels. Today, we have extensive experimental toolboxes to explore the role of ion channels in physiological signaling and disease. These approaches have aided our understanding of noncardiac forms of excitable cell disease. Most importantly, beyond fundamental scientific discovery, findings in our generation have aided in the diagnosis and treatment of disease in patients of all ages and backgrounds. In our current era, not a week goes by where new data link familial and acquired forms of heart failure and arrhythmia with gene variants in membrane proteins. Since the initial findings of Keating and others, thousands of articles have illustrated molecular pathways underlying congenital human arrhythmia. In fact, for Brugada Syndrome alone, hundreds of articles have been published illustrating that >200 mutations in 20 genes are linked with the arrhythmia.2

Although we must continue to embrace the search for new variants and novel arrhythmia pathways, we are entering a new era where translation to new therapies, whether surgical, pharmacological, or molecular are paramount. As we seek new methodologies to treat heart disease, we are faced with data that simple, single cell-based assays or culture models may not address on their own.

We certainly champion the merits of approaches that deeply explore cell and molecular mechanisms of arrhythmia. But it is also reasonable to step back and note that we easily forget that the heart is in a chest, is highly innervated, and contains a host of nonmyocytes vital for cardiac function. We may assume that global molecular and immunoblot analyses of the heart measure transcript and protein of only contracting myocytes, but tend to lose focus on the integration of these molecules and their partners on neighboring smooth muscle cells, fibroblasts, and intracardiac nerves. Certainly, understanding the collective relationship between these disparate cell types is central to understand the arrhythmia substrate and its triggers. These relationships are dynamic and change with development, physical, and environmental stresses, and certainly in the face of cardiovascular and noncardiovascular pathologies.

The nonreductionist approach, a systems approach, started well before our years in university. Although some cardiac cell types were known, the careful work of Sir Thomas Lewis and others on mechanisms of arrhythmias spanned many years in the early twentieth century. For research then, in the absence of genetic/protein information, everything centered on the relationship of the animal, the heart, and the outcome (arrhythmia). These years spawned the development of breakthroughs in antiarrhythmic pharmacological agents, for example, propranolol, many forms of which are still in use today. Furthermore, this era ushered in the use of ablation and device-based therapies to treat atrial and ventricular arrhythmias. In fact, mechanisms of arrhythmias were more clearly defined in isolated, whole hearts with the development of digital, multiplexing mapping of several hundred sites on the heart simultaneously. Similar techniques are used today in arrhythmia ablations.

This lead to more pharmacopeia with drugs being developed to affect specific ion channels and electrophysiology as studied in normal hearts.3 We do not argue the merits of understanding the impact of compounds on normal myocardium. However, generally drugs are given to patients with diseased hearts. We then launched into the remodeled heart phase discovering that what we assumed about the diseased heart was certainly incomplete. The diseased heart is not normal, thus understanding the disease heart also requires a new understanding of not only sick myocytes but also the complex remodeling relationship between contracting myocytes and other cell types noted above (eg, altered cardiac innervation). Furthermore, the remodeled substrate cannot always be
pigeonholed (eg, the evolving substrates of atrial fibrillation). In fact, most remodeled substrates are rather dynamic because there are always cells being remodeled and those that are beginning the process of reverse remodeling.

We have made immense progress in our goal to treat arrhythmia, however as illustrated with the examples below, we must continue to bridge reductionist approaches with their impact on not only cardiac but also organism function.

For example, population genome-wide association studies have reported links between variants in SCN10A and parameters of conduction (PR/QRS intervals) perhaps providing a hint as to the mechanism of a patient’s cardiac conduction block. Subsequent studies emerged (including from one of our laboratories) testing the effects of a Nav1.8-specific blocker on cardiac cell electrophysiology. The Boyden laboratory focused on I_{to}, the single canine Purkinje cell, whereas Roden and Veldkamp laboratories on single rabbit and murine myocytes. Boyden/Veldkamp both determined that there was no A-803467-sensitive current in single cardiac cells studied, whereas Roden laboratory explained that late Na current that was blocked by A-803467 explained the abnormal QRS and PR interval findings. The Veldkamp group determined that it was the functional presence of Nav1.8/SCN10A in intracardiac neurons that in fact played an important role in the cardiac cell electric activity. By working at the single cardiomyocyte level, we may have missed this discovery. However, our understanding of the complexities of arrhythmia is certainly still in its infancy. In this case, the story is further complicated in that the enhancer in SCN10A physically contacts both SCN5A and SCN10A to alter SCN5A expression. Certainly, not simply a cardiac single cell or single molecule disorder! As far as we are aware, therapies for these patients at risk have not been altered based on these fundamental findings.

Combining their unique location and the existence of some proteins that differ from ventricular muscle, Purkinje cells with genetic lesions will have a large impact on cardiac rhythm. One example is seen in idiopathic ventricular fibrillation (VF). In 1 study, 5 patients with idiopathic VF were studied as they all had short coupled premature beats that initiated VF. During preablation mapping, early signals from the Purkinje network were clear at the onset of VF. Ablation at this site was successful. A molecular follow-up of these patients showed that a chromosomal haplotype-producing increased transcript levels of DPP6 (dipeptidyl peptidase-like protein 6) may be associated with familial VF. Subsequent work suggested that DPP6 was rich in Purkinje myocytes and coexpression of DPP6 with Kv4.3 (I_{to}, alpha subunit) enhanced I_{to}. Thus, a gain-of-function in DPP6 potentially mediated an electric effect on I_{to} to shorten repolarization setting the stage for the arrhythmia. But the question remains whether all of these arrhythmias are cardiac cell in origin? I_{to} currents are found in various neurons (A-type current), and DPP6 is also auxiliary protein to neuronal Kv channels and implicated in autism disorders and amyotrophic lateral sclerosis.

A proband has been described with a similar arrhythmia. Notably, a variant in a novel splice form of DPP6 (termed DPP6T) was identified. In coexpression work (in noncardiac cells), the DPP6T variant increased Kv4.3 currents augmenting I_{to}. But does this occur in both cardiac myocytes and nonmyocyte cells of the patient? Sturm et al showed the effects of I_{to}-based therapies on arrhythmia susceptibility in this patient. Importantly, a combination of dalfampridine (4-aminopyridine, among others a blocker of I_{to} in cardiac cells), cilostazol (PDE3 [phosphodiesterase 3] inhibitor), and low-dose quinidine reduced proband’s VF events >90 fold and ECG returned to normal. Nevertheless with the reasoning of the specific cardiac cell type thought to be involved and the underlying genetic lesion, personalized I_{to} therapy proved to be antiarrhythmic in this patient. But are all effects observed in the patient because of the effects of the drugs on cardiac cell I_{to} currents? How do we know? Can we improve these approaches? Perhaps one answer lies in new targeted genome editing platforms (CRISPR/Cas). Although exciting progress has been made using in vivo targeted gene replacement approaches, it has not yet been broadly implemented in arrhythmia. Do we know enough to do this? For example, do we need to target simply cardiac cells or a selection thereof? Or do we need to better understand all cell types necessary or sufficient for the arrhythmia.

In summary, the findings of Keating and colleagues revolusionized the field of cardiovascular arrhythmia genetics—setting the stage for 2 decades of work that propelled our understanding of disease mechanisms and potential antiarrhythmia therapies. Moving forward, for the advent of new disease treatments, it will be critical that we continue to integrate our understanding of animal and organ physiology with the complex molecular pathways that govern the myriad of cardiac cell types. We must rededicate ourselves to integrating these approaches to find therapies that (1) work in the heart, but more importantly (2) effectively integrate the likely complex multisystem phenotypes found in the patient. The best future scientists should be able to see the arrhythmia as the dynamic interplay of multiple molecular pathways, cells and systems, thus treating the patient, not simply the cell.

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None.

**References**


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