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The ever shrinking world of cardiac ion channel remodeling: The role of microRNAs in heart disease

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Precordial palpitation, developed by the ancient Greeks, gave the first observation of the normal rhythm of the heart. Since that time, understanding the substrate for this rhythm and how this substrate is regulated in heart disease has been an ever expanding scientific endeavor. The targets, on the other hand, are getting smaller and smaller. Whole heart studies have fallen to electrophysiological studies of channels involved in generating the action potential. In turn, the understanding of individual protein subunits of the channels became the standard of excellence. The discovery that changes in protein levels were not always correlated with changes in gene activity (RNA production) indicates that the time to dig deeper has arrived. In this edition of *Heart Rhythm*, Girmatsion et al¹ do exactly that, finding that changes in a small regulator, a microRNA, lead to the big problem of atrial fibrillation (AF).

Atrial fibrillation is the most common cardiac arrhythmia, affecting anywhere from 1% (under 60 years of age) up to 7% (over 80 years of age)² of the population. Medical intervention has been, to date, somewhat poor in treating AF, largely due to the complexity of the disorder that comes, in part, from the fact that AF is not homogeneous in its pattern of emergence or in its underlying triggers. In some cases, individual AF events with short durations may be asymptomatic. In other cases, the presence of initial AF events predisposes patients to more persistent AF, leading to the understanding that “AF begets AF”³. The underlying triggers for the initiation of AF are not clear, although there is a predisposition for AF with age, alcohol consumption, and the presence of other underlying cardiovascular pathologies such as myocardial infarction or hypertension⁴. What is clear is that changes in the ion currents, due to remodeling of sarcolemmal ion channels and gap junctions, occur over time in AF and lead to formation of an arrhythmogenic substrate and the perpetuation of AF⁵. Although many authors have speculated that understanding either single channel function and/or the trafficking of channels to the myocyte membrane will allow us to treat most, if not all, of the ion channel based cardiac diseases, these hypotheses have yet to lead to clear pharmacological targets for AF, likely due to the myriad of “off target” effects many pharmacological agents have in the atria. This lack of a promising pharmacopeia for AF has led researchers to dig even deeper into the cell in an attempt to understand the events that cause loss of ion channel function. As with many cases in science, the deeper we dug, the more we found. Now, as shown by Girmatsion et al¹, we are faced with understanding yet another layer of ion channel regulation, the regulation by microRNAs.

MicroRNAs were first described in 1993 when Lee and colleagues published their discovery of a novel RNA molecule that produced very small RNA fragments of 22 and 61 nucleotides⁶. Their studies in *Caenorhabditis elegans* (*C. elegans*) described the 22 nucleotide “lin-4” that had inhibitory activity against another RNA, lin-142. In 2001 several publications noted that these “microRNAs” (miRs), as they were now called, were an extensive class of molecules with the potential to regulate vast numbers of cellular processes^{7–9}. In 2002 it was shown that while miRs were highly conserved across phylogeny, there is tissue specificity including 34 different miRs that are specific to the heart¹⁰. miRs are made within the nucleus of cells, where they are produced from a longer pri-miRNA that is directly transcribed from the introns of protein-coding genes or transcribed from polycistronic genes forming a nucleotide stem loop. The pri-miRNA is then processed to miRNA by an RNase, Drosha, which leaves the free stem loop as the pre-miRNA. After export from the nucleus to the cytoplasm, Dicer cleaves the pre-miRNA into a duplex consisting of two miRNA molecules, one destined to be the mature miRNA and the other (the miRNA*, formed in the nucleus from the opposite side of the stem loop) destined to be lost to degradation. This duplex is then further processed in the RNA-induced silencing complex (RISC) of the cells with degradation of the miRNA*, leaving behind a mature inhibitory miRNA, more commonly known as a miR. These small molecules then suppress the availability of other RNA for transcription to protein either by translational repression or cleavage of mRNA within the cell (for full reviews see references 11 and 12).

The heart functions with minute by minute adjustments of the cellular environment in order to rapidly accommodate changing cardiovascular needs of the body. Tight regulation of the action potential requires that myocytes are capable of either increasing or decreasing ion channel function in the face of these rapidly changing environments. Prior to the discovery of miRs, studies on the regulation of ion channel function focused on ion channel function at the sarcolemma, ion channel trafficking to the sarcolemma or on the production of ion channel subunits at the level of gene transcription^{13,14}. These methods of regulation can be slow, with the genes being quiescent until signal transduction pathways activate transcription factors which then act upon individual genes or set of genes to produce ion channel transcripts. These transcripts then need to undergo translation and trafficking to the membrane prior to causing a functional impact on the cell. Controlling of the number of ion channels that make it to the sarcolemma by regulating the trafficking of the protein subunits also takes time, as chaperone molecules need to be either increased or decreased in order to alter the flow of subunits to the membrane. Regulation of channel function by post-translational modifications such as phosphorylation/dephosphorylation can provide minute to minute regulation but is not always highly efficient potentially leaving residual channel function. Therefore none of these regulatory mechanisms is sufficient for the rapid control of ion channel function that is needed to maintain normal action potentials in the face of cellular stress. On the other hand, the activity of the miRs can regulate ion channels by decreasing RNA availability for transcription of the ion channel subunits. Loss of these subunits would lead to a rapid loss of functional channels, giving a mechanism for molecular changes under physiological or pathological stress.

In normal heart, miRs regulate a number of cellular functions including developmental programs such as promoting stem cells to commit to the cardiomyocyte lineage, normal cellular growth and right ventricular development and septation¹⁵. Studies looking at panels of miRs in heart disease have shown that miRs are dramatically altered several pathological conditions known to lead to ventricular arrhythmias¹⁵. One study found that miR-1 is increased in ventricular tissue following myocardial infarction and went on to identify two miR-1 targets; Kir2.1 which is the channel responsible for the inward rectifier K⁺ current (IK₁) and Connexin43 the primary gap junction protein in the ventricle¹⁶. These channels, which underlie maintenance of conduction velocity in the heart, were both decreased in ventricular tissue in the presence of increased miR-1. These authors went on to show that in an animal

model where miR-1 was artificially increased in the ventricle, Kir2.1 and Cx43 were lost and the hearts showed slowed conduction and a propensity for arrhythmias¹⁶. These data indicate that miR-1 regulates Kir2.1 and Cx43 levels in ventricular myocytes and that alteration in miR-1 levels in pathology may be a trigger for the production of the arrhythmogenic substrate. In this edition of *Heart Rhythm*, Girmatsion et al¹ focused on the role of miR-1 in the atria and perhaps surprisingly they found the opposite effect than was seen in AF as compared with ventricular arrhythmias. Rather than an increase in miR-1 in this arrhythmia, there was a decrease in miR-1 with a subsequent reciprocal increase in Kir2.1. Interestingly, neither of the major atrial connexins (Cx43 or Cx40) was changed, suggesting the potential for differential regulation of these ion channels by miR-1 in atrial vs. ventricular myocytes. This differential response to fibrillation in different heart components indicates that fibrillation is not a simple electrical pathology. Instead it is a disease of the molecular landscape of the heart with molecular heterogeneity between compartments underlying the individual tissue response to arrhythmogenic triggers.

To date, it has not been clear how this heterogeneity is established or what regulates the molecular landscape in cardiac pathology. With the finding the miRs play a role in ion channel regulation, and more interestingly the finding that differential regulation of miRs occurs in different heart compartments, helps us understand how this molecular heterogeneity is formed. It is tempting to now think that this opens the door for more specific drug targets and potential for developing that missing pharmacopeia for AF. Unfortunately to do this we have one more question to answer. What regulates the miRs?

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