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REGENERATIVE MEDICINE (SM WU, SECTION EDITOR)

Using iPSC Models to Probe Regulation of Cardiac Ion Channel Function

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Abstract

Purpose of Review Cardiovascular disease is the leading contributor to mortality and morbidity. Many deaths of heart failure patients can be attributed to sudden cardiac death due primarily to ventricular arrhythmia. Currently, most anti-arrhythmics modulate ion channel conductivity or β-adrenergic signaling, but these drugs have limited efficacy for some indications, and can potentially be proarrhythmic.

Recent Findings Recent studies have shown that mutations in proteins other than cardiac ion channels may confer susceptibility to congenital as well as acquired arrhythmias. Additionally, ion channels themselves are subject to regulation at the levels of channel expression, trafficking and post-translational modification; thus, research into the regulation of ion channels may elucidate disease mechanisms and potential therapeutic targets for future drug development.

Summary This review summarizes the current knowledge of the molecular mechanisms of arrhythmia susceptibility and discusses technological advances such as induced pluripotent stem cell-derived cardiomyocytes, gene editing, functional genomics, and physiological screening platforms that provide a new paradigm for discovery of new therapeutic targets to treat congenital and acquired diseases of the heart rhythm.

Keywords iPSCs · Arrhythmia · High-throughput · Ion channels · Disease modeling

Introduction

Cardiovascular disease (CVD) is the leading contributor to mortality and morbidity. However, development of drugs in this area of medicine is steadily declining [1]. CVD is among the indications with the highest attrition during drug development and the lowest rates of regulatory approval. Moreover, the complexities of discovering new therapeutic targets for heart disease have caused several major pharmaceutical companies to refocus their efforts away from the field. The underlying reasons are diverse and range from early scientific or clinical risk, to commercial risks when approved [2, 3•, 4]. To improve the success rate of new drugs for CVD, new modalities to identify and validate therapeutic agents are paramount for addressing the needs of the large and growing population of patients with CVD [5].

Induced pluripotent stem cell (iPSC) technology is an exciting new platform for modeling CVDs that provides a virtually limitless source of iPSC-derived cardiomyocytes (iPSC-CMs) from individual patients harboring congenital disease-causing mutations [6]. The technology provides a clear advantage over primary rodent cardiomyocytes in that the hiPSC-CMs retain human genetic and cellular context. Recent developments in high-throughput assays to rapidly quantify the kinetics of excitation-contraction coupling enable screening of chemical libraries for functional genomics and drug development applications. We anticipate that these technical advances will revolutionize disease modeling and drug discovery for CVD, particularly, diseases of heart rhythm. This review focuses on the molecular mechanisms of heart rhythm disorders and on the opportunity of using iPSC models to explore the influence of non-ion channel mechanisms to the development of cellular

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arrhythmia and the possibility to discover new classes of drug targets for clinical development.

Heart Rhythm Disorders and Current Therapeutic Strategies

Clinically, electrocardiography measures the instantaneous change in electrical field potential to infer the direction, magnitude, and duration of electrical activity during each cardiac cycle. Myocardial excitation is initiated by the sinoatrial (SA) pacemaker cells which activate the atria and then propagates through the cardiac conduction system to enable synchronized excitation and contraction of each ventricle. At the cellular level, each depolarization and repolarization cycle, known as an action potential (AP), initiates excitation-contraction (EC) coupling linking the electrical membrane potential to myosin-actin cross-bridge formation (Fig. 1). An AP is generated when the membrane potential reaches a supra-threshold voltage that activates voltage-gated sodium channels to rapidly depolarize the membrane, known as phase 0 of the AP. Phase 1 of the AP is a brief repolarization period mediated by two transient outward potassium currents, $I_{to,f}$ and $I_{to,s}$. Phase 2 of the AP is maintained by the influx of Ca^{2+} ions through voltage-gated L-type calcium channels (LTCCs, I_{Ca}). Towards the end of phase 2, the efflux of K^+ ions is conducted by voltage-gated potassium channels (I_{Kr} and I_{Ks}) to continue repolarization during phase 3 of the AP. The resting state, phase 4, is maintained by multiple mechanisms. The sodium-potassium ATPase utilizes ATP to actively extrude Na^+ in exchange for influx of K^+ that maintains the Na^+ and K^+ gradients. Additionally, the inwardly rectifying potassium current (I_{K1}) is activated at hyperpolarized membrane potentials to maintain the resting potential.

Dysfunction of impulse generation or aberrant conduction through the heart can cause abnormal heart rhythms. The SA node has a faster intrinsic rate than the atrioventricular node and therefore normally dictates heart rate. However, if other cardiac sites show enhanced automaticity, they may generate competing stimuli and induce arrhythmia. Such triggers are observed as early or delayed after depolarizations (EADs and DADs) at the cellular level (Fig. 1). Myocardial damage or abnormal electrophysiological properties may allow an electrical impulse to encounter conduction block to one region, propagate along an alternative path to then re-activate the original region, a condition known as reentry that can lead to premature and/or repeated reactivation of the atria or ventricles. Clinically, arrhythmias are managed pharmacologically with anti-arrhythmic drugs that either target ion channels directly or inhibit β-adrenergic signaling. However, current anti-arrhythmics have limited efficacy and can sometimes be proarrhythmic [7].

Genetic Basis for Arrhythmia Disorders

Unraveling the molecular mechanisms of human genetic disorders has yielded profound insights into normal as well as pathological human physiology. Many variants in predominantly ion channel proteins have been identified as being the

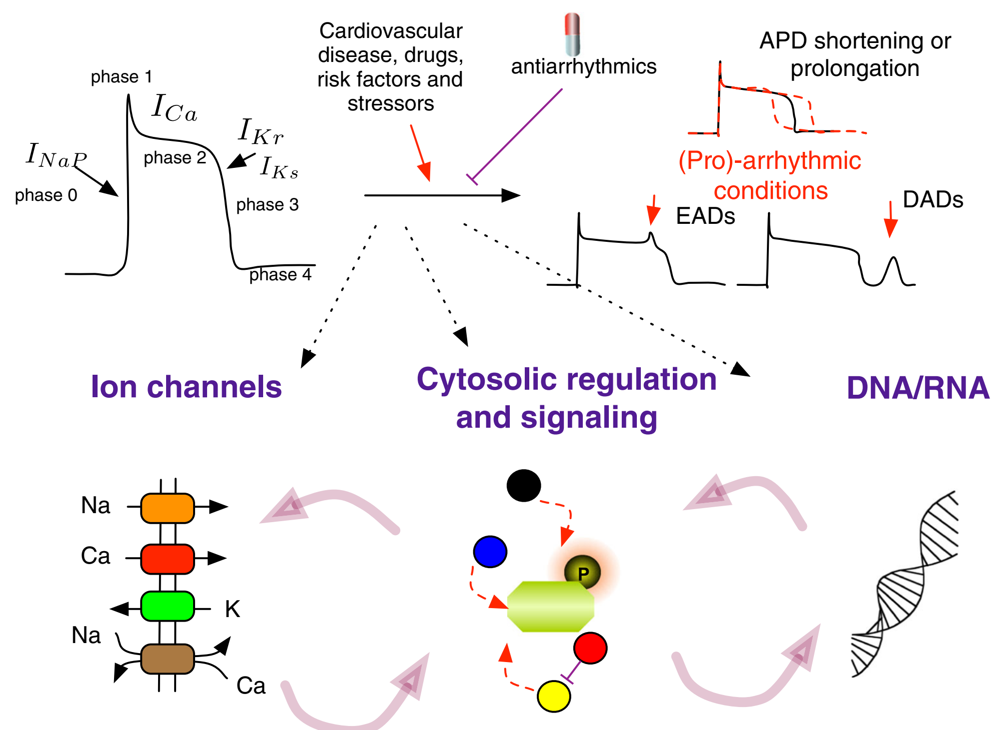

Fig. 1 Electrophysiological properties of cardiomyocytes are altered in congenital as well as acquired arrhythmic diseases and are responsible for increased susceptibility to arrhythmia and sudden cardiac death. Gene defects, differential expression from modulation at either the levels of gene expression or translation, aberrant signaling, and protein modifications can underpin the electrophysiological disease state

causative or a modifying factor for arrhythmic disease (Table 1), including long QT syndrome (LQTS) [8], short QT syndrome (SQTS) [9], catecholaminergic polymorphic ventricular tachycardia (CPVT) [10], sick sinus syndrome (SSS) [11], atrial fibrillation (AFib) [12, 13], and Brugada syndrome (BrS) [14]. With a prevalence of 1% in the general population and 6% in people older than 65, AFib is the most common type of arrhythmia. However, the pathophysiology of AFib is complex as a patient's genetic background can be both causative (familial AFib) and potentially act as a disease modifier (non-familial AFib) [13]. Similarly, many polymorphisms have been identified by genome-wide association studies (GWAS) that map to genes associated with arrhythmic diseases and may contribute to additional risk albeit with small effect sizes [15–18]. In contrast, LQTS is the most common type of congenital arrhythmia with a prevalence of 1:2000 [19]. Taken together, there are many genetic variants associated with distinct proarrhythmic diseases that can be mined to gain additional insights into molecular mechanisms.

Congenital arrhythmia is typically caused by mutations affecting the biophysical properties of the pore-forming α-subunit of voltage-gated ion channels. However, biophysically normal α-subunit variants with hindered (a) synthesis or trafficking, (b) recycling and internalization, or (c) degradation pathways may also be disease causing, as ion channel density also determines current profiles [20]. Mutations affecting the pore-forming subunit, such as *KCNQ1* (LQTS1, SQTS2), *KCNH2* (LQTS2, SQTS1), and *SCN5A* (LQTS3, BrS), may cause heritable heart rhythm disorders. Importantly, it has become clear that variants in ancillary proteins, ranging from β-subunits to enzymes, may also cause or contribute to congenital arrhythmia. Both *KCNE1* (LQTS5) and *KCNE2* (LQTS6), which encode for β-subunits that modulate the conductance I_{Kr} and I_{Ks}, have been shown to be causative for LQTS by slowing repolarization [21–23]. Mutations in cytoskeletal and scaffolding proteins have also been linked to arrhythmia. LQTS4 is caused by a mutation in ankyrin-β (*ANKB*) [24]. *ANKB* mutations reduce the trafficking and expression of the Na^+/K^+ ATPase and Na^+/Ca^{2+} exchanger as well as alter intracellular calcium handling that sensitizes the cells to arrhythmia [25]. Caveolae, which create small invaginations of the cell membrane, form micro-domains for ion channel expression and regulation [26, 27]. Mutations in *CAV3* were shown to cause LQTS9 by increasing late sodium current [28] and modulating the pacemaker current I_f [29].

In addition to ion channels and ion channel-modifying proteins, intracellular calcium-handling proteins have profound effects on the electrophysiological properties of cardiomyocytes, and aberrant regulation of these genes can cause arrhythmia susceptibility. CPVT is an inherited arrhythmogenic disease caused by mutations in *RYR2* [30, 31], calsequestrin (*CASQ2*) [32, 33], calmodulin (*CALM*) [34], *TECRL* [35], or triadin (*TRDN*) [36, 37]. RYR2 is the sarcoplasmic reticulum (SR)-anchored ligand-gated ion channel responsible for calcium flux from the SR to the cytosol to initiate contraction. SR calcium leak through RYR2 can induce arrhythmia when the leaked calcium is able to induce an ectopic action potential. Many other proteins, including TRDN, CALM, and CASQ2, associate with RYR2 and are involved in functional regulation of RYR2 or are responsible for calcium buffering in the SR. Hence, defects in the function of these proteins may adversely affect SR calcium physiology and render a patient susceptible to arrhythmia.

Mutations in the enzyme *GPD1L* were shown to cause BrS2 by modulating PKC-dependent phosphorylation of SCN5A resulting in reduced I_{Na} [38–40]. Interestingly, mutations in the enzyme *TECRL* were also linked to CPVT [35]. However, the precise function of this protein remains unknown, but a role in lipid metabolism is suspected. Moreover, variants in the hormone *NPPA* [41] and multiple transcription factors have also been implicated in AFib [42, 43]. Such variants likely act through modulation of ion channels transcription, atrial remodeling, pulmonary vein development, or development of cardiac conduction system [44, 45]. Taken together, defects not only in pore-forming α-subunits of ion channels, but also in a wide variety of other proteins have been shown to cause or modulate susceptibility to arrhythmia.

Ion Channel Regulation and Implications for Disease

Sudden cardiac death due to arrhythmia is the leading cause of mortality in patients with heart failure [46]. Altered ion channel expression and regulation as well as tissue damage are thought to underpin arrhythmic susceptibility in the failing heart. Cardiomyocytes in the failing heart have prolonged AP duration, decreased repolarization reserve, and a high rate of Ca^{2+}-dependent arrhythmias resulting from electrical remodeling due to aberrant neurohormonal signaling. Understanding the regulatory mechanisms of ion channel function and how these are perturbed by CVDs may yield possibilities for drug development.

The electrophysiological properties of cardiomyocytes are heavily regulated by intrinsic signals, extrinsic signals, and mechanical stress in both healthy and diseased patients [47]. Several neurohormonal pathways are active in the heart and are responsible for adverse remodeling and progression of the disease [48, 49]. Ca^{2+}-dependent signaling regulates transcriptional activity in the heart via CaMK2 [50–52]- and calcineurin [52, 53]-dependent pathways. In addition, LTCCs provide the cell with the ability to indirectly sense membrane voltage changes and alter gene expression as its C-terminal domain translocates to the nucleus to regulate gene expression [54]. miRNAs fine-tune expression of multiple genes by preventing translation of the target mRNA, and several

Table 1 Overview of gene variants associated with arrhythmia

Protein type	Proteins and diseases
Ion channels (pore-forming α-subunits)	- Na+ ○ *SCN5A* (I_{Na}, Nav1.5): LQTS3, BrS1, SSS1, AFib10, ATRST1, VF1, PFHB1A, SIDS ○ *SCN10A*: AFib - K+ ○ *KCNQ1* (I_{Ks}, Kv7.1): LQTS1, JLNS1, SQTS2, AFib3 ○ *KCNH2* (I_{Kr}, hERG): LQTS2, SQTS1, BrS8, AFib ○ *KCNA5* (I_{Kur}, Kv1.5): AFib7 ○ *KCNJ2* (I_{K1}, Kir2.1): LQT7, SQTS3, AFib9 ○ *KCNJ5*: LQT13, AFib ○ *KCNJ8*: BrS9, AFib, SIDS ○ *KCND3* (I_{to}, Kv4.3): BrS13, AFib ○ *KCNN3*: AFib - Na+/K+ ○ *HCN4* (I_f): BrS14, SSS2 - Ca2+ ○ *CACNA1C* (I_{CaL}, Cav1.2): LQT8, BrS3, TS ○ *CACNA2D1*: BrS10 ○ *CACNA2D4*: AFib ○ *RYR2*: CPVT1, AFib ○ *TRMP4*: BrS16 - Cation (multi) ○ *TRPM4*: PFHB1B
Ion channels (accessory subunits)	- Na+ ○ *SCN1B*: BrS5, AFib13 ○ *SCN2B*: BrS17, AFib14 ○ *SCN3B*: BrS7, AFib16 ○ *SCN4B*: LQTS10, AFib17 - K+ ○ *KCNE1*: LQTS5, JLNS2, AFib ○ *KCNE2*: LQTS6, AFib4 ○ *KCNE3*: BrS6, AFib ○ *KCNE4*: AFib ○ *KCNE5*: BrS12, AFib ○ *ABCC9*: AFib12 - Ca2+ ○ *CACNB2*: BrS4, AFib
Gap junctions	*GJA1* (AFib), *GJA5* (AFib11, ATRST1)
Scaffolding proteins	*ANKB* (LQTS4), *CAV3* (LQTS9, SIDS), *SNTA1* (LQTS12), *AKAP9* (LQTS11), *LMNA* (AFib), *GREM2* (AFib), *NUP155* (AFib15), *JPH2* (AFib), *SYNE2* (AFib)
Regulatory proteins	*CALM1* (LQTS14/CPVT4), *CALM2* (LQTS15), *CALM3* (CPVT), *TRDN* (CPVT5), *CASQ2* (CPVT2), *RANGRF* (BrS11), *SLMAP* (BrS15), *PRKAG2* (WPWS)
Signaling molecules	*NPPA* (AFib6, ATRST2)
Transcription factors	*GATA4/5/6* (AFib), *NKX2.5/2.6* (AFib), *ZFHX3* (AFib), *PITX2* (AFib), *TBX5* (AFib)
Enzymes	*GPD1L* (BrS2), *TECRL* (CPVT3)

Sources: UniProt, OMIM, and [8–14]

LQTS long QT syndrome, *SQTS* short QT syndrome, *CPVT* catecholaminergic polymorphic ventricular tachycardia, *SSS* sick sinus syndrome, *AFib* atrial fibrillation, *BrS* Brugada syndrome, *WPWS* Wolff-Parkinson-White syndrome, *ATRST* atrial standstill, *VF* familial paroxysmal ventricular fibrillation, *PFHB* progressive familial heart block, *JNLS* Jervell and Lange-Nielsen syndrome, *TS* Timothy syndrome, *SIDS* sudden infant death syndrome

miRNAs modulate cardiovascular physiology or are differentially expressed in heart disease [55–60]. Alternative splicing of *SCN5A* resulting in truncated non-functional channels is increased in heart failure [61]. In addition, multiple ion channels undergo differential expression in the failing human heart: *KCNJ2*, *KCND3*, *CACNA1C*, *SCN5A*, *KCNH2*, *KCNQ1*, *RYR2*, *CASQ*, *SERCA2A*, *NCX*, Na+/K+ ATPase, and calmodulin are reduced, whereas *HCN4* (I_f) is increased [62], albeit with some heterogeneity and inconsistency across studies. These findings likely contribute to the AP prolongation, automaticity, and conduction disturbances observed in the failing heart.

Abnormal trafficking may be an important contributing factor to altered ion channel function in diseased hearts.

However, the mechanisms of anterograde and retrograde trafficking of membrane proteins leading to the highly organized membrane structure of the cardiomyocyte remain poorly understood [63]. Drugs such as probucol and fluoxetine can cause drug-induced QT prolongation by blocking hERG channel trafficking [64]. In addition, hERG membrane expression is regulated by extracellular potassium concentration, as reduction of extracellular potassium leads to hERG internalization and degradation, and hence reduced I_{Kr} [65]. This mechanism may in part explain why hypokalemia is a risk factor for arrhythmia and sudden cardiac death in heart disease [66]. miR-1 is upregulated in patients with coronary artery disease and infarcted rat hearts [67], and overexpression of this miRNA induces arrhythmia in mice by downregulating trafficking genes [68]. Gap junctions, comprising connexin channels, play an important role in electrical conduction of the electrical excitation throughout the heart. In the healthy heart, gap junctions are localized at the intercalated discs for longitudinal spread of excitation. However, the expression of GJA1 is reduced at both the mRNA and protein level and improperly localized in heart failure [69, 70]. These deficits may contribute to conduction disturbances and proarrhythmia seen in heart failure.

Post-translational modifications (PTMs) enable transcription-independent modulation of cellular function, typically by inducing conformational changes. Phosphorylation is arguably the most studied reversible enzymatic PTM. Phosphate groups can be added to serine, threonine, and tyrosine residues by kinases, and removed by phosphatases. The human genome encodes for more than 500 kinases and many have been shown to play a critical role in heart disease [71]. Phosphorylation events orchestrated by PKA and CaMK2 participate in the excitation-contraction coupling as well as mediate the chronotropic, inotropic, and lusitropic effects of β-adrenergic stimulation on the heart [72]. PKA has many targets in the heart, including phospholamban, LTCCs, RYR, and troponin I [73, 74]. Similarly, CaMK2 has also been implicated in the phosphorylation of several ion channels in cardiomyocytes, such as sodium channels, LTCCs, RYR, and SERCA [75]. However, CaMK2 hyperactivity has been implicated in the pathogenesis of heart failure and arrhythmia [76]. Phosphorylation of sodium channels by CaMK2 increases late sodium current [77], while phosphorylation of RYR2 increases channel open probability leading to SR calcium leak and reduced SR calcium content [78]. As a result, inhibition of CaMK2 was recognized as a potential therapeutic strategy, for which industry has initiated a search for pharmaceutical grade compounds although none have yet reached clinical testing yet [76, 79].

Many other PTMs or amino acid modifications have been shown to modulate ion channel function and may have a role in disease. Glycosylation regulates multiple steps of protein biogenesis including protein folding, trafficking, and function [80]. Many voltage-gated channels are heavily glycosylated, and terminal sialic acid glycosylation plays an important role in voltage sensing and gating of ion channels [81]. Glycosylation-associated genes are differentially expressed in the heart with respect to chamber specification and developmental state [82]. Moreover, targeted deletion of such glycosylation-associated genes has profound effects on cardiac excitability. Aberrant glycosylation or sialylation of substrates, including calsequestrin [83], was also observed in CVD [84]. A missense mutation in calsequestrin (K206N) introduced an additional glycosylation site resulting in a larger molecular weight protein that reduced calcium-binding properties and caused arrhythmia by dysregulating calcium handling [85]. Protein methylation of sodium channels has also been observed on arginine residues implicated in Brugada syndrome and LQTS3 [86]. However, the mechanism by which methylation affects sodium channel function remains poorly understood. S-acylation is a reversible covalent fatty acid modification of cysteine residues and is enzymatically mediated by acyltransferases and is reversed by acyl-protein thioesterases. S-palmitoylation is the most common form of S-acylation [87, 88]. Palmitoylation motifs were shown to regulate late sodium current, and a mutation of a single cysteine (C981F) has been associated with susceptibility to arrhythmia [89]. Ubiquitinylation and SUMOylation are known to affect ion channel function by targeting the protein for degradation or manipulating activity [90]. SUMOylation was shown to modulate activation and inactivation kinetics of KCNQ1 (I_{Ks}) and KCNA5 (I_{Kur}), an atrial repolarizing potassium channel [91, 92]. In addition, a missense mutation attenuating deSUMOylation of TRPM4 associated with heart block resulted in impaired endocytosis and elevated cell membrane density [93]. Finally, oxidative stress is often associated with disease, including HF and AFib, and also enables protein modification and alteration of function [94, 95]. CaMK2 can be directly modified by reactive oxygen species leading to sustained activation and increased late sodium current, Na^+/Ca^{2+} overload, and arrhythmogenesis [96, 97]. Oxidative stress has been linked to calcium channel [94] and potassium channel dysfunction [98]. In particular, KCNA5 is modified with sulfenic acid residues in response to oxidative stress in AFib leading to reduced surface expression and current density without altering the biophysical properties of the channel [99].

In summary, the biogenesis and function of cardiac ion channels is a tightly regulated process that is critical for the normal function of the heart. Ion channels are regulated at many levels from transcription and mRNA splicing to protein trafficking and post-translational modification. The results of genomic studies have identified many variants in regulatory genes of ion channels, and the emergence of new technologies to study the influence of these mutations in healthy and diseased states presents an exciting opportunity for the discovery of novel regulatory networks for future drug discovery.

iPSC-CMs for Arrhythmic Heart Disease Modeling

The ability to study the intricate mechanisms governing cardiomyocyte excitability in human cardiomyocytes has been extraordinarily difficult due to the scarce availability of human myocardial samples, and the inability to isolate and propagate primary human cardiomyocytes. Classical models to measure cardiovascular electrophysiology have been limited mostly to heterologous expression of α-subunits in cell lines, such as HEK293 and CHO that do not retain the cardiomyocytes context and rodent models that do not recapitulate the human cellular context. The development of human iPSCs as a stem cell source [100] and efficient protocols for deriving cardiomyocytes [101, 102] has enabled the routine production of virtually unlimited quantities of human iPSC-CMs. iPSC-CMs make it possible to assess the integrated effect of genetic variants or external stimuli on excitation-contraction coupling. Additionally, recent advances in gene-editing tools facilitate the generation of de novo mutations in a healthy patient background or correction of a putative disease-causing mutations in patient lines [103, 104]. As a result, iPSC-CMs have been utilized as a model to study monogenic CVDs and to address potential roles of putative at-risk alleles identified by GWAS [105]. Multiple familial arrhythmogenic disorders have been studied using iPSC-CMs, including LQTS [106, 107], CPVT [108], arrhythmogenic right ventricular dysplasia [109], and more recently BrS [110]. Initially, the focus was mainly channel α-subunit-dependent channelopathies, whereas more recently, the contribution of alternate mechanisms to arrhythmia has also been probed with iPSC-CMs, such as Cav3 in LQTS9 [111]. While this area remains understudied, we believe that iPSC-CMs can aid in deconvoluting complex mechanisms of arrhythmia, as these cells allow the investigation of the interaction between multiple molecular cascades in the context of a human cardiomyocyte.

Despite their key advantages, iPSC-CMs have limitations such as variability, impurity, physiological immaturity, and lack of chamber specificity, which we hope further research will resolve [112]. iPSC-CMs are considered immature counterparts for the human adult cardiomyocytes, with respect to their structure, metabolism, and electrophysiology [113]. For example, the slightly depolarized resting membrane potential and the lack of reliance on sodium current for AP generation may restrict the use of such immature cells to probe the contribution of late sodium current to arrhythmia [114•]. However, considerable progress has been made using culture media alteration and tissue engineering strategies to further purify and mature the physiology of the iPSC-CMs [115–118]. In addition, iPSC-CMs can be directed to ventricular or atrial fate by defined factors [119] and can hence be used to study cell-autonomous AFib mechanisms. Importantly, several companies have been founded to generate commercial grade human iPSC-CMs to satisfy the needs of industry.

High-Throughput iPSC-CM Models for Drug Discovery and Toxicity Screening

iPSC-CMs have considerable benefits for drug discovery as well, which remain to be explored, as these cells enable screening with physiological readouts compared to traditional target-based screens of ion channel α-subunits. This feature represents a significant advance for drug discovery by moving away from target-centric approaches and towards modulation of a physiological phenotype that is a result of many complex biological processes working in concert, such as APs or Ca^{2+} transients (Fig. 2). Physiological screens with iPSC-CMs have the following benefits: (1) the function of the channels can be probed in context with other ion channels and regulatory proteins that more closely resemble in vivo environment; (2) disease states can be recreated using iPSC-CMs derived from specific CVD patients; and (3) the contribution of non-ion channel proteins and cell signaling can be studied using powerful screening tools such as functional and chemical genomics approaches.

Currently, mainly low-throughput assays have been used to probe iPSC-CMs as well as animal-derived cardiomyocytes. Recent developments in high-throughput optical platforms to record AP kinetics enable the ability to screen chemical libraries and quantify physiological parameters [120]. These methods may truly revolutionize cardiovascular drug discovery. Optically, recording of APs overcomes the inherent throughput limitations of single-cell patch clamping electrophysiology assays, as cells can be imaged in multi-well plates rather than as single cells. Additionally, the reduced cost of using voltage-sensitive dyes provides an advantage over more expensive approaches such as microelectrode arrays that may be prohibitively costly for functional genomics applications. Organic small molecule calcium and voltage dyes are available with appropriate fluorescent properties to enable high-throughput screening [114•, 121, 122]. The high-throughput quantification of cardiomyocyte contractility is more challenging. Many assays use cellular displacement as a surrogate for contractility, since the direct measurement of force is more difficult to accomplish at a micrometer scale [123, 124]. For example, traction force microscopy (TFM) on single cells and on micro-patterned gels [115], micropost deflection assays [125], or engineered heart tissues [126] can be used to measure force, but difficulties in fabrication of suitable devices as well as development of instruments to measure force in a high-throughput fashion have limited the widespread adoption of these tools.

There is also considerable interest to use iPSC-CMs to study the cardiovascular safety of drug candidates [127–129]. A traditional approach to determine the proarrhythmic liability of a given drug candidate has been to measure inhibition of hERG (I_{Kr}) channels. Although measuring I_{Kr} inhibition has kept potentially toxic drugs off the market, the technique does not predict Torsade de Points (TdP) as some drugs inhibit I_{Kr}

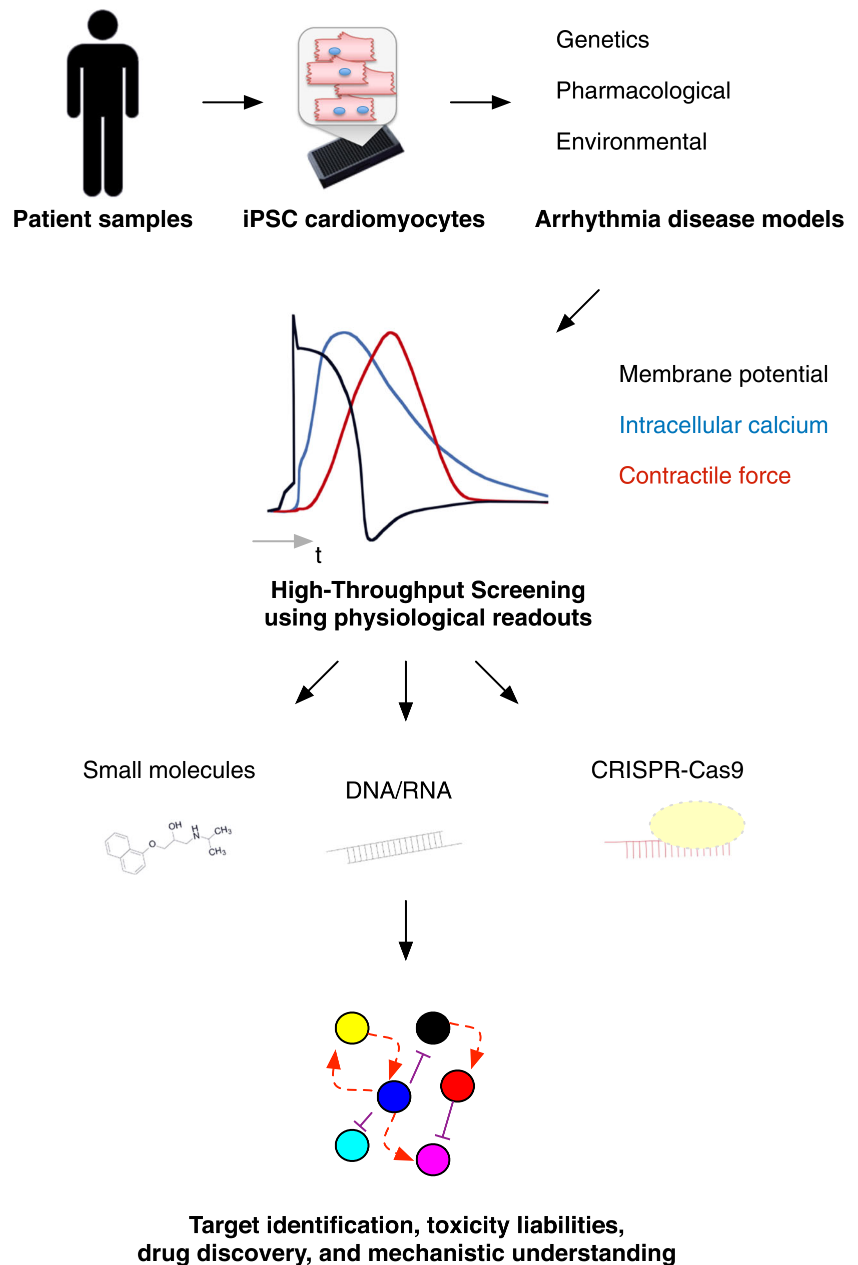

Fig. 2 Induced pluripotent stem cells can be derived with high efficiency from patient tissue samples and efficiently differentiated to virtually all cell types of the body, including cardiomyocytes (iPSC-CMs). iPSC-CMs can be utilized to study mechanisms of arrhythmia dependent on extrinsic (pharmacological drugs and chemical environment) as well as intrinsic factors (genetics). While traditional techniques for quantifying electrophysiological parameters and excitation-contraction coupling are low throughput, recent developments in both screening technology and high-throughput assays enable phenotypical screens of moderate sized chemical libraries (small molecules, nucleic acids, or CRISPR/Cas9) in parallel. We believe these technologies will drive our current understanding of molecular arrhythmia mechanisms further and will revolutionize target identification and drug discovery for anti-arrhythmics in addition to determining of cardiotoxicity liabilities of drug candidates

without causing TdP [130, 131] and other drugs cause TdP without inhibiting I_{Kr} [132–134]. Given the phenotypical screening potential of iPSC-CMs, these cells can be utilized for toxicity screening without bias towards the underlying mechanisms. The utility of iPSC-CMs for prediction of cardiotoxicity is currently being investigated by the FDA under the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative. The project aims to develop a more predictive model for TdP by bringing together academia, pharmaceutical industry, and regulatory agencies to facilitate the adoption of a new paradigm for assessment of drug toxicity. Current CiPA studies support the utility of iPSC-CMs for proarrhythmic testing; however, further development and validation are likely warranted before adoption [135•, 136•, 137•]. Additionally, proarrhythmia is not the only potential liability of drug candidates as some drugs have been shown to induce cardiomyopathy and heart failure [138]. iPSC-CMs can be similarly applied for such toxicities using other phenotypic readouts. Sharma et al. [128] recently analyzed the cardiotoxic propensity of 21 anti-cancer kinase inhibitors by developing a risk index based on the relationship between plasma C_{max} and the dose that affected both contractility and viability in vitro in iPSC-CMs. The results correlated well with clinically reported adverse events in patients.

iPSC-CMs represent a flexible platform for screening of diverse chemical collections that probe the contribution of both coding and non-coding mechanisms to arrhythmia. By combining genetic, pharmacological, or environmentally

induced iPSC-CMs models of disease with physiological screens using libraries of small molecules [139], (anti-) miRNAs, siRNAs, and/or Cas9 guide RNAs [140, 141], the role of individual proteins or signaling pathways on the cardiomyocyte excitability can be rapidly tested (Fig. 2). For example, by knocking down individual kinases, the impact of signaling cascades on arrhythmia propensity can be probed directly under normal or diseased conditions. With CRISPR gene-editing technology, individual PTM sites, such as for phosphorylation or ubiquitinylation, can be mutated at the genomic level to study the role of specific PTM events on arrhythmia propensity. As these edited proteins remain under endogenous promotor regulation, this methodology is superior to traditional viral overexpression. Taken together, iPSC-CMs offer the ability to conduct phenotypical screening assays on human cardiomyocytes, which provides relevant new avenues for drug discovery, both for conducting screens for rescuing disease phenotypes and for quantifying cardiotoxic liabilities.

Conclusion

Arrhythmia is a significant disease burden and the development of novel drugs for this indication has stagnated in recent years. It is becoming increasingly clear that multiple proteins in addition to ion channels may confer susceptibility to arrhythmia. Additional research into the regulation of ion channel expression, trafficking, and regulation is needed and may provide novel directions for drug development. New technologies such as iPSC-CMs, gene editing, functional genomics, and high-throughput screening platforms provide new approaches to our search for novel arrhythmia therapies and, hopefully, will lead to the discovery of new drugs to alleviate arrhythmogenesis in patients with CVDs.

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Compliance with Ethical Standards

Conflict of Interest Arne A. N. Bruyneel, Wesley L. McKeithan, Dries A. M. Feyen, and Mark Mercola declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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