

## REVIEW ARTICLE

# Elucidating arrhythmogenic right ventricular cardiomyopathy with stem cells

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**Abstract**

Human stem cells have sparked many novel strategies for treating heart disease and for elucidating their underlying mechanisms. For example, arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart muscle disorder that is associated with fatal arrhythmias often occurring in healthy young adults. Fibro-fatty infiltrate, a clinical hallmark, progresses with the disease and can develop across both ventricles. Pathogenic variants in genes have been identified, with most being responsible for encoding cardiac desmosome proteins that reside at myocyte boundaries that are critical for cell-to-cell coupling. Despite some understanding of the molecular signaling mechanisms associated with ARVC mutations, their relationship with arrhythmogenesis is complex and not well understood for a monogenetic disorder. This review article focuses on arrhythmia mechanisms in ARVC based on clinical and animal studies and their relationship with disease causing variants. We also discuss the ways in which stem cells can be leveraged to improve our understanding of the role cardiac myocytes, non-myocytes, metabolic signals, and inflammatory mediators play in an early onset disease such as ARVC.

**KEYWORDS**

arrhythmia, ARVC, cardiomyopathy, cytokines, desmosomes, gene mutations, stem cells

## 1 | INTRODUCTION

Human stem cells have created many new opportunities for treating heart disease and for elucidating their underlying mechanisms. Treatment strategies leverage, largely, the medicinal features of stem cells for repairing the diseased heart. However, significant clinical application, to date, has not been realized. In contrast, the pluripotency of stem cells has been leveraged to create an in vitro phenocopy of the heart (i.e., “heart in a dish”) that is especially useful for understanding mechanisms of inherited disorders. A classic example is that for Long QT

syndrome where induced pluripotent stem cells (iPSC) obtained from a patient's skin sample were used to differentiate cardiac myocytes that express the same disease-causing variant and, importantly, the patient's electrophysiological phenotype (Itzhaki et al., 2011). Utilizing stem cells this way, cellular and molecular mechanistic links between disease causing variants and clinical phenotypes can be elucidated (Kim et al., 2013; Liang et al., 2016; Novak et al., 2012). Herein, we focus on another inherited cardiac disorder, arrhythmogenic right ventricular cardiomyopathy (ARVC) that can occur early in life, is difficult to diagnose, has variable expressivity,

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and has an unknown molecular mechanism of arrhythmia that stem cells may help uncover.

ARVC is an inherited heart muscle disorder that is associated with fatal arrhythmias, and it is one form of a larger group of arrhythmogenic cardiomyopathies (ACM) (Maron et al., 2006). In ARVC, sudden cardiac arrest or death often occurring with exercise can be the initial presentation in otherwise healthy young adults (Basso, Corrado, Marcus, Nava, & Thiene, 2009). When it was first described by Marcus et al. (Marcus et al., 1982), fatty replacement in the heart wall was originally thought to be a congenital abnormality limited to the right ventricle (RV). It is now recognized to be a progressive disease process that can involve the left ventricle (LV) as well (Corrado et al., 1997). Importantly, exercise is not just associated with arrhythmia occurrence, but high intensity and endurance training seem to promote both the expression of the phenotype and the disease progression (James et al., 2013; Rojas & Calkins, 2015). Due to its early onset, ARVC has recently been recognized as an important cause of death in the pediatric population (Te Riele, James, Calkins, & Tsatsopoulou, 2021). Pathogenic or disease-causing variants have been identified. The majority (60%) of these mutations encode the desmosome proteins plakophilin-2 (PKP2), plakoglobin (JUP), desmoglein-2 (DSG2), desmoplakin (DSP), and desmocollin-2 (DSC2) (Hall et al., 2018). The cardiac desmosome is a cell-to-cell adhesion structure present at the intercalated disc that is essential for coupling cardiac myocytes together. Rare variants have also been reported for desmosome and nondesmosome proteins including Desmin (DES), Luma (TMEM43), and transcription growth factor  $\beta$ -3 (TGF $\beta$ 3) (Lorenzon et al., 2017). The prevalence of ARVC in the general population is estimated to be 1 in 1000 to 1 in 5000 (Basso et al., 2009; Groeneweg et al., 2015). Recently, it was shown in a multinational clinical study that among patients with definite ARVC, approximately 11% will have a life-threatening arrhythmia within 6 years of diagnosis (Cadrin-Tourigny et al., 2021). A common observation in patients with ARVC is the occurrence of ventricular arrhythmias in the absence of any significant structural heart disease, corresponding to an early, concealed, phase (Ingles et al., 2018). However, as the disease advances, myocyte apoptosis, inflammation, fibrosis, and accumulation of myocardial fibro-fatty infiltrate become evident (Corrado et al., 1997), which is an important clinical hallmark of the disease. Given the high risk of sudden cardiac death, implantable cardioverter-defibrillators are often recommended and both catheter ablation and anti-arrhythmic medications are frequently used to treat recurrent ventricular arrhythmias. In this review article, we begin by focusing on arrhythmia mechanisms in

ARVC based on clinical and animal studies and their relationship to disease causing variants, and then we describe how stem cells can be leveraged to understand the mechanistic underpinning of ARVC phenotypes.

## 2 | ARVC ARRHYTHMIA MECHANISMS BASED ON CLINICAL AND ANIMAL STUDIES

An appealing rationale for arrhythmia in ARVC patients is that fibro-fatty infiltrate electrically isolates neighboring myocytes and causes slow and blocked impulse (i.e., action potential) propagation, which is a requirement for reentrant arrhythmias. Electrical isolation also unloads neighboring myocytes, which can facilitate triggered arrhythmias such as premature ventricular complexes (PVCs). Taken together, the heightened substrate for reentry combined with an increased burden of triggers is a perfect storm for fatal arrhythmias. Indeed, studies in humans with ARVC demonstrated initiation of ventricular tachycardia by ventricular ectopy produced by catecholamine infusion (Philips et al., 2013), which is consistent with the clinical presentation of sudden death in ARVC patients during exercise. Interestingly, even though PVC burden is mechanistically related to arrhythmia, ablation of PVCs in patients with ARVC was recently shown to be largely ineffective (Assis et al., 2021), whereas ablation of VT has some success (Bai et al., 2011), suggesting a deeper understanding is necessary for more effective treatments.

Genetically modified mouse models have been instrumental in helping to understand the relationship between desmosome protein variants and ARVC clinical phenotypes. Early studies were able to recapitulate certain phenotypes but not all, highlighting the variable expressivity associated with ARVC. For example, in a transgenic mouse model with cardiac-restricted overexpression of the DSP C-terminal mutation (R2834H), structural abnormalities including enlargement of both ventricles, cardiomyocyte apoptosis, cardiac fibrosis, desmosomal dysfunction, and lipid accumulation were reported (Yang et al., 2006). In the same study, cardiac mechanical dysfunction was also noted; however, despite these abnormalities no indication of arrhythmia (a key phenotype) was mentioned. Alternatively, heterozygous PKG-deficient mice exhibit increased RV volume, reduced RV function and, importantly, reduced RV impulse conduction times, and ventricular arrhythmias including ectopy and tachycardia. Interestingly, no fibro-fatty replacement of the RV myocardium or reduced gap junction protein connexin43 (responsible for electrical connections between ventricular myocytes cells) expression

was observed (Kirchhof et al., 2006). In contrast to these studies, a more complete and severe phenotype including premature death, arrhythmia, ventricular dysfunction, and fibro-fatty infiltrate were demonstrated in a mouse model of TMEM43 (Padron-Barthe et al., 2019) and in a DSP knockout model (Lyon et al., 2014). The severity of disease expression in these models is mirrored by clinical observations in ARVC patients with DSP (Smith et al., 2020) and TMEM43 (Merner et al., 2008) mutations. Crucially, mouse models have also demonstrated that environmental factors, such as exercise, can worsen the disease. For example, AAV-mediated introduction of the PKP2 R735X mutation into sedentary mice had no effect on RV function or histology during a 10-month follow-up. However, exercise training in these mice caused impaired RV function and connexin 43 delocalization at myocyte gap junctions (Cruz et al., 2015). Likewise, in heterozygous PKG deficient mice (Kirchhof et al., 2006) and cardiomyocyte-specific DSP-deficient mice (Lyon et al., 2014), ventricular arrhythmias worsened with exercise. To summarize, while some mouse models are not a complete and faithful phenocopy of the human disease, in aggregate they replicate the important phenotypes of ARVC, and they underscore the broad spectrum of the disease observed in patients.

Mouse models have also deepened our understanding of the cellular and molecular mechanisms of arrhythmia associated with desmosome protein variants. In a PKP2 gene haploinsufficiency mouse, dysfunction of ion channels known to interact with desmosome proteins ( $I_{Na}$ ) and ultrastructural abnormalities were observed despite normal connexin 43 expression. Interestingly, flecainide was required to obtain slow impulse conduction and arrhythmia (Cerrone et al., 2012). In a PKP2 cardiac restricted knockout mouse, significant RV abnormalities were observed along with isoproterenol induced arrhythmias and a disruption of intracellular calcium signaling (Cerrone et al., 2017), which in a subsequent study was associated with calcium abnormalities and calcium sparks that would favor ventricular ectopy (Kim et al., 2019). Finally, in mice with cardiac overexpression of mutant DSG2-N271S, intercalated disc (desmosomes/adherens junctions) abnormalities and lower  $I_{Na}$  current density were observed suggesting interaction between DSG2 and Na(V)1.5 (Rizzo et al., 2012). Importantly, these mouse studies provide a better mechanistic understanding of why reentry and ectopy are heightened in patients with ARVC.

Despite the benefits of genetic mouse models of ARVC and rare naturally occurring ARVC in animals (as in Boxer dogs; Basso et al., 2004), important limitations exist. For example, in some animal models arrhythmia occurrence is

rare or requires a challenging agent (e.g., Flecainide) in the absence of any significant structural remodeling, which is unlike that in humans and suggests other factors may be contributing to arrhythmogenesis during the early onset concealed phase. Moreover, whether a genetically modified mouse model can faithfully phenocopy the human disease may depend on how genes are modified (e.g., conditional, inducible, knockout, knockin, etc.), which can affect the severity of the phenotype and if any compensatory changes in related genes or off target genetic expression occurs. Furthermore, mouse models of disease remain largely unproven for finding clinically feasible therapy. In part, because mice can lack specificity to humans for some ARVC mutations (Padron-Barthe et al., 2019; Stroud et al., 2018). These limitations may explain why some important clinical features of ARVC remain unexplained, such as why fatal arrhythmias have an early onset in the absence of significant structural disease (i.e., concealed phase) and the exact role of fibro-fatty infiltrate and exercise in arrhythmogenesis (Rojas & Calkins, 2015).

### 3 | MOLECULAR SIGNALING ASSOCIATED WITH ARVC VARIANTS

The mechanistic relationship between desmosome protein variants associated with ARVC and arrhythmogenesis is not obvious. This is unlike other inherited arrhythmia syndromes like Long QT syndrome or catecholaminergic polymorphic ventricular tachycardia (CPVT), where a mutation associated with an ion channel or calcium regulatory protein can be directly tied to arrhythmia substrates. For example, in Long-QT type 2 syndrome, a mutation in the hERG channel, which provides repolarizing current, is reduced. This prolongs action potential duration (APD) which is known to cause triggered arrhythmias due to early afterdepolarizations (January, Gong, & Zhou, 2000). In contrast, the molecular signaling associated with ARVC mutations portrays a complex scenario. Most studies have associated desmosome mutations with suppression of Wnt/GSK3 $\beta$ / $\beta$ -catenin signaling pathways (Lorenzon et al., 2017), which regulate a wide range of physiology and pathophysiology during development and adulthood. Wnt (a family of ligand proteins) binds to its membrane receptor protein and causes the accumulation of  $\beta$ -catenin in the cytoplasm that, eventually, translocates to the nucleus. In the nucleus,  $\beta$ -catenin is the signal transducer of canonical Wnt signaling through T cell/lymphoid-enhancing binding (Tcf/Lef) transcription factors that activate myogenesis and inhibit adipogenesis (Maeda et al., 2004). In the absence of Wnt binding to its receptor,  $\beta$ -catenin is destroyed in the cytoplasm, which

involves glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ) (Clevers & Nusse, 2012). How is this related to ARVC phenotypes? JUP (i.e., plakoglobin), also known as  $\gamma$ -catenin, is a closely related homologue of  $\beta$ -catenin. Importantly, it is believed that  $\gamma$ -catenin activates transcription factors much less efficiently than  $\beta$ -catenin leading to suppression of canonical Wnt signaling and enhanced adipogenesis, fibrogenesis, and myocyte apoptosis. Mutations affecting other desmosome proteins (e.g., DSP) can also promote nuclear localization of JUP/ $\gamma$ -catenin leading to Wnt/ $\beta$ -catenin suppression and enhanced expression of adipogenic and fibrinogenic genes (Garcia-Gras et al., 2006). A similar, yet slightly different mechanism may occur with TMEM43 variants (e.g., TMEM43-S358L), which does not disrupt integrity and localization of desmosomal proteins but instead interferes with AKT signaling and activates GSK3 $\beta$  which then inhibits  $\beta$ -catenin-dependent transcription (Padron-Barthe et al., 2019; Rajkumar, Sembrat, McDonough, Seidman, & Ahmad, 2012). It has also been shown that silencing and activation of GSK3 $\beta$  mitigates and accelerates, respectively, ARVC features in DSG2 mutant mice, indicating that aberrant GSK3 $\beta$  signaling contributes to the pathogenesis of ARVC (Chelko et al., 2016). Despite this understanding, how these abnormal signaling pathways are related to ventricular arrhythmias and sudden cardiac death in patients with ARVC is not clearly delineated.

#### 4 | THE FIBRO-FATTY INFILTRATE IN ARVC

Fibro-fatty infiltrate, which can present as fibrosis with varying degrees of fat (adipose tissue), is a hallmark of ARVC and includes infiltrative cells (e.g., adipocytes, lymphocytes) and their secretions (e.g., cytokines), along with fibrotic tissue (e.g., collagen) (Basso et al., 1996; Corrado et al., 1997; Haliot et al., 2021). Obviously, an upregulation of adipogenic signaling associated with ARVC variants (as describe above) would promote a fibro-fatty infiltrate, but why/where does it originate from and what is its contribution to arrhythmogenesis? To begin, it has been estimated that in the normal heart there are more nonmyocytes than myocytes (Zhou & Pu, 2016), so the fibro-fatty infiltrate associated with ARVC is possibly intrinsic. It has been shown that adipocytes in ARVC originate from progenitor cells in the second heart field (RV) (Lombardi et al., 2009). Moreover, evidences from electroanatomic substrate mapping (Garcia, Bazan, Zado, Ren, & Marchlinski, 2009) and autopsies (Basso et al., 1996) suggest the disease progresses from epicardium

to endocardium. Similarly, mouse studies have shown that epicardium-derived cells are the main cell type contributing to the infiltrate associated with ARVC (TMEM43 in particular) (Padron-Barthe et al., 2019). Several studies suggest that the mesenchymal stem cell (MSC), a stromal progenitor cell, may be a precursor for fibro-fatty infiltrate (Sommariva et al., 2016). Cardiac MSCs exist, and MSCs are very sensitive to Wnt signaling, so any disruption could promote MSCs to differentiate into adipocytes (Ross et al., 2000). Importantly, MSCs express desmosome proteins and those harboring ARVC variants demonstrate alterations in cytoskeletal organization, and bone marrow derived MSCs display increased proliferation rate and migrate to the heart (Scalco et al., 2021). Therefore, cells extrinsic to the heart may also be contributing to the fibro-fatty infiltrate. Thus far, there is strong evidence that MSCs are a source of fibro-fatty infiltrate associated with ARVC, and that MSC function can be impacted by ARVC variants just as myocytes are.

On one hand, the presence of fibrosis and/or a fibro-fatty infiltrate can impact cardiac mechanical function resulting in ventricular chamber dilation with reduced contractility. These structural changes also provide an anatomical substrate for arrhythmias similar to other disease processes that result in an increased risk of arrhythmia due to scar such as myocardial infarction (MI). However, ventricular arrhythmias in ARVC often occur in pediatric patients early in the disease process and, unlike MI, are not always reproducibly induced at electrophysiological study suggesting that other drivers of arrhythmia vulnerability are present in ARVC. For example, the secretome of the cells that comprise the fibro-fatty infiltrate could contribute to arrhythmogenesis. Cardiac MSCs exist and have a broad secretome that does remodel in disease, as we (Sattayaprasert et al., 2020) and others (Monsanto et al., 2017) have shown. This secretome includes cytokines that are known to significantly impact ion channels (Monnerat et al., 2016), calcium regulation (Zuo et al., 2020), and gap junction proteins (Lazzerini et al., 2019), just to name a few. It is also worth noting that certain cytokines can dislodge desmosome proteins (Asimaki et al., 2011), which could interfere with canonical Wnt signaling and contribute to the ARVC phenotype as described above. It has been well established that inflammatory cytokines are increased in the serum and heart tissue of ARVC patients (Asimaki et al., 2011; Campian et al., 2010; Corrado et al., 1997). Moreover, in vitro models have shown that DSG2 mutations can cause aberrant cytokine expression (Hawthorne et al., 2021). Similarly, in several ARVC variants activation of NF $\kappa$ B, a master regulator of inflammation that is linked to GSK3 $\beta$  is upregulated in cardiac myocytes and its inhibition mitigates abnormalities of intercalated disk

proteins, myocyte apoptosis, inflammatory cytokines, LV contractile dysfunction, and the ECG (Chelko et al., 2019). Importantly, the study by Chelko et al. (Chelko et al., 2019) suggest that the innate immune response in myocytes may be sufficient to create the ARVC phenotype. In sharp contrast, TMEM43 is sporadic in myocytes and mostly expressed in nonmyocytes in mice and human heart tissue (Stroud et al., 2018), suggesting that nonmyocytes may be playing a primary role for some ARVC mutations (Liu, Chen, & Shou, 2018). Finally, it is certainly possible that some of the fibro-fatty infiltrate arises systemically, because bone marrow MSCs expressing desmosome mutations have been found in the heart (Scalco et al., 2021), and excessive exercise is associated with increased levels of inflammatory cytokines (da Rocha et al., 2019). It is, therefore, clear that the fibro-fatty infiltrate can arise from within and beyond the heart, and its impact on arrhythmia substrates may be much more than simply structural.

## 5 | STEM CELL MODELS FOR STUDYING ARVC

Exactly how ARVC variants in desmosome proteins are causally related to arrhythmogenesis in patients is still largely unknown, despite the availability of genetic mouse models and a better understanding of the underlying molecular mechanisms. Stems cells, such as iPSCs that can be derived from a patient's blood or skin sample (Takahashi & Yamanaka, 2006), may help address these gaps in knowledge. For example, by utilizing iPSCs, the genetic complexity of an individual patient can be maintained *in vitro*, which is not possible using gene editing techniques (e.g., CRISPR). Additionally, cells of almost any type (e.g., cardiac myocytes, MSCs, adipocytes, etc.) can be differentiated, allowing the investigation of multiple cell types (e.g., co-cultures) that are completely specific to humans. Finally, the number of cells that can be differentiated are almost unlimited and can be cultured as a syncytium in monolayers (Lee et al., 2012) or more realistic tissue constructs (Blazeski et al., 2019). Such assays are essential for studying the molecular and electrical basis of complex arrhythmia phenotypes because they allow concomitant study of intracellular and extracellular signaling alongside electrophysiological mapping. These features of iPSCs may prove particularly useful for ARVC because, in part, species differences can be circumvented, and it is possible to study key components (e.g., myocytes and fibro-fatty infiltrate) independently.

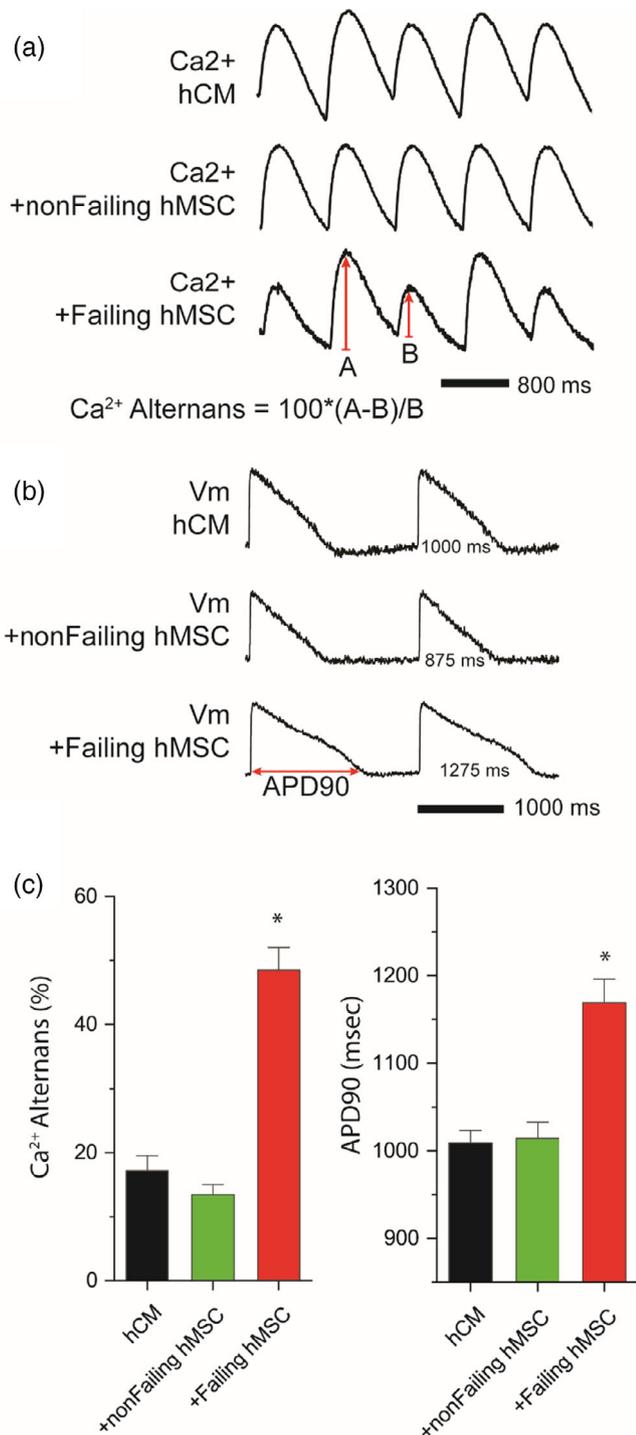
To date, most ARVC studies that have utilized iPSCs for investigating arrhythmia mechanisms have focused on the myocyte, despite the potential role of nonmyocytes

(i.e., fibro-fatty infiltrate). In one of the first studies to utilize human iPSCs, cardiac myocytes with a PKP2 disease causing variant were derived and shown to have decreased  $\beta$ -catenin as expected (see above); however, this was not sufficient to reproduce anything resembling clinical ARVC phenotypes. Only when the same cardiac myocytes were exposed to adult-like metabolism and pro-adipogenic factors were ARVC phenotypes observed, including exaggerated lipogenesis, apoptosis, and calcium-handling abnormalities (Kim et al., 2013). This study and another with similar findings (Ma et al., 2013) suggest that the fibro-fatty infiltrate contributes significantly to the ARVC phenotype. Notably, when myocytes with a pathological PKP2 variant are cultured as a functional syncytium that supports impulse propagation, reduced connexin-43 was observed and field potentials exhibited a prolonged rise time suggesting abnormal impulse conduction (although not tested) (Caspi et al., 2013). As Kim et al. (Kim et al., 2013) observed, adipogenic stimuli worsened the phenotype (Caspi et al., 2013). As with mice, completely knocking out PKP2 in iPSC derived human cardiac myocytes show a more severe phenotype as evidenced by slow impulse conduction velocity (CV) (Dou et al., 2020); however, knocking out PKP2 may overestimate the effects of the disease-causing variant. Variable expressivity is also evident with other ARVC mutations expressed in iPSC derived myocytes. For example, with a DSG2 variant, increased pro-inflammatory cytokine expression was noted along with shorter action potentials and abnormal calcium regulation. It is interesting to note that impulse CV associated with this DSG2 variant was similar to controls, and only when DSG2 was knocked out were any changes observed (Hawthorne et al., 2021). We have also found in preliminary studies no difference in impulse CV between myocytes derived from human control iPSCs and myocytes derived from human iPSCs with the TMEM43 p.Ser358Leu disease causing variant (Vasireddi et al., 2019). It is possible that iPSC-derived cardiac myocytes used in more mature engineered tissue constructs would enhance ARVC phenotypes (Blazeski et al., 2019). Nevertheless, it seems as though when ARVC mutations are studied in myocytes derived from patient specific iPSCs, it is still difficult to explain arrhythmogenesis, even in a monogenetic disorder like inherited ARVC.

## 6 | FIBRO-FATTY INFILTRATE VERSUS THE MYOCYTE IN ARVC

We contend that for ARVC and other cardiac disease (e.g., heart failure), the inflammatory secretome of

nonmyocytes remodels and can regulate arrhythmia substrates (Sattayaprasert et al., 2020). Shown in Figure 1 is an example of the effect human cardiac MSCs have on



**FIGURE 1** Shown are calcium (Ca<sup>2+</sup>) alternans and APD measured in normal human cardiac myocytes (hCM) under normal conditions when co-cultured with cardiac hMSCs from nonfailing hearts (nonfailing hMSCs) and failing hearts (Failing hMSC). Example traces in Panel a show Ca<sup>2+</sup> alternans and APD (APD90) in Panel b. Summary data are shown in Panel c. \**p* < .002 versus hCM

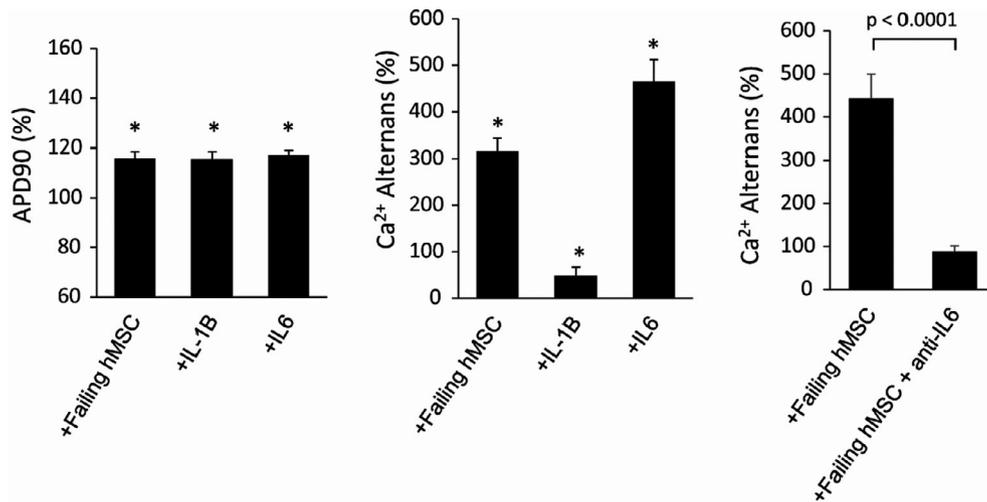
arrhythmia substrates measured in normal human cardiac myocytes (hCM) derived from iPSCs. In these experiments, cardiac myocytes were co-cultured for 2 days with MSCs isolated from nonfailing or failing human hearts. Co-cultures were treated with fluorescent indicators for measuring calcium transients and action potentials utilizing optical mapping techniques (McPheeters, Wang, Werdich, Jenkins, & Laurita, 2017). Calcium transients measured from cardiac myocytes during steady state stimulation exhibit much larger beat-to-beat alternans of calcium transient amplitude when co-cultured with MSCs from failing hearts compared to nonfailing hearts (Figure 1, Panel a). Previously, calcium transient alternans has been mechanistically linked to arrhythmias (Pruvot, Katra, Rosenbaum, & Laurita, 2004) and are prevalent in heart failure (Wilson et al., 2009). We also found that APD during steady state stimulation was much longer when co-cultured with MSCs from failing hearts compared to nonfailing hearts (Figure 1, Panel b). Like calcium alternans, prolongation of APD is also a well described arrhythmia substrate in human heart failure (Glukhov et al., 2010). In numerous studies by others, similar changes in calcium alternans and APD have been attributed to changes in calcium regulatory protein expression (Laurita, Katra, Wible, Wan, & Koo, 2003) and ion channel remodeling (Li, Lau, Ducharme, Tardif, & Nattel, 2002); however, this is unlikely to explain our results (Sattayaprasert, Nassal, Wan, Deschenes, & Laurita, 2016).

Given that human cardiac MSCs remodel in disease, we tested the possibility that changes in the secretome of MSCs from failing hearts could explain the changes in arrhythmia substrates we observed. Specifically, we found that MSCs isolated from failing hearts have increased secretion of IL-6 and IL-1 $\beta$  compared to MSCs isolated from normal hearts (Sattayaprasert et al., 2020). As shown in Figure 2, when myocytes alone were exposed to IL-1 $\beta$  or IL-6, APD increased (left panel), but only IL-6 increased calcium alternans (middle panel). Finally, when failing cardiac MSC and myocyte co-cultures were treated with anti-IL-6, calcium alternans (unique to IL-6) was rescued. Notably, we also found that normal MSCs can be anti-arrhythmic and suppress calcium alternans (Sattayaprasert et al., 2016) by IGF-1 secretion (Sattayaprasert et al., 2020). These results suggest that novel therapies that inhibit (e.g., IL-6) or mimic (e.g., IGF-1 pathway activation) specific cytokines may be effective at treating arrhythmias associated with heart failure or ARVC.

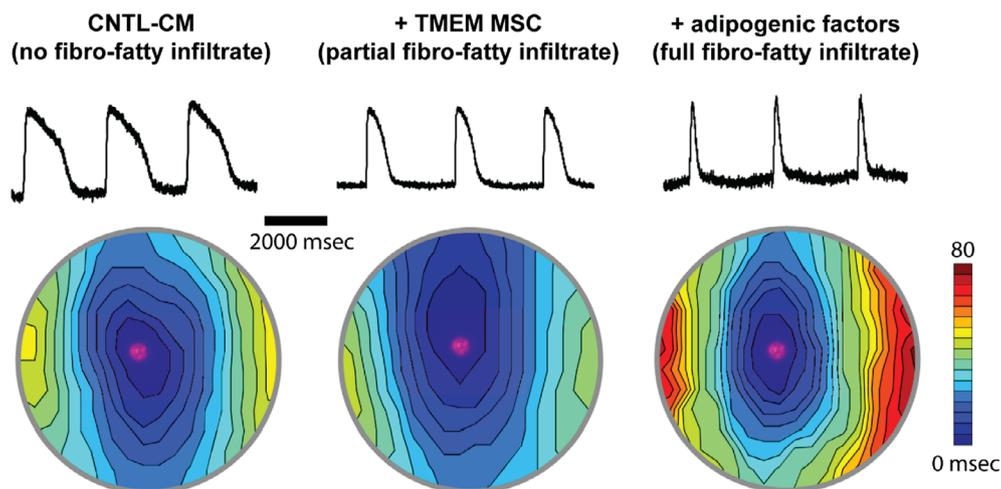
It is possible that the fibro-fatty infiltrate from ARVC patients can regulate arrhythmia substrates just like MSCs isolated from failing hearts can, but not necessarily in the same way. Testing this, however, by obtaining

fresh human heart samples from ARVC patients would be extremely difficult. Nevertheless, in preliminary studies (Vasireddi et al., 2019) we sought to identify the separate effects of the fibro-fatty infiltrate and myocytes with ARVC mutations. As already mentioned, we observed no difference in CV between myocytes derived from normal patients and myocytes from a patient with a TMEM43 disease causing variant (p.Ser358Leu), suggesting the fibro-fatty infiltrate is crucial. To test this, we compared iPSC derived cardiac myocytes from a normal patient

with the same myocytes co-cultured with either TMEM43 MSCs (partial fibro-fatty infiltrate) or TMEM43 MSCs plus adipogenic factors (full fibro-fatty infiltrate). Cells were cultured as monolayers on aligned nano-fiber multi-well plates to create a more mature (anisotropic) alignment of cell structure (Vasireddi et al., 2018) from which impulse CV and APD could be measured using optical mapping techniques, as we have described previously (McPheeters et al., 2017). As shown in Figure 3, we found that TMEM43 MSCs (partial fibro-fatty infiltrate) have



**FIGURE 2** IL-1 $\beta$  and IL-6 effect on arrhythmia substrates. All measurements are a percent of that measured in normal myocytes alone. Panel a shows the effect of IL-1 $\beta$  ( $n = 6$ ) and IL-6 ( $n = 5$ ) on average APD90 (left) and calcium (Ca<sup>2+</sup>) alternans (middle), when administered to normal cardiac myocytes alone. Levels of significance are compared to normal myocytes where \* $p < .001$  (for Ca<sup>2+</sup> alternans,  $n = 23$ ), \* $p < .02$  (for APD90,  $n = 6$ ). Panel on the right shows the rescue of Ca<sup>2+</sup> alternans induced by failing hMSCs ( $n = 18$ ) with anti-IL-6 ( $n = 18$ ) treatment. Levels of significance are compared with normal myocytes alone ( $n = 12$ ) where \* $p < .001$



**FIGURE 3** Influence of TMEM43 MSCs with (full fibro-fatty infiltrate) and without (partial fibro-fatty infiltrate) pro-adipogenic factors on normal cardiac myocytes. Example action potentials and conduction maps are shown above for normal myocytes (CNTL-CM), CNTL-CM + TMEM MSC, and CNTL-CM + TMEM MSC + pro-adipogenic factors. The full fibro-fatty infiltrate (TMEM MSC + pro-adipogenic factors) significantly reduce APD and conduction velocity (crowding of isochrone lines)

little effect on CV and APD compared to control myocytes alone; however, only when TMEM43/myocyte co-cultures were exposed to adipogenic factors (full fibro-fatty infiltrate) was slow impulse CV (crowding of isochrones) and APD shortening observed. These preliminary results highlight the importance of the fibro-fatty infiltrate including metabolic and inflammatory mediators, and they demonstrate how stem cells could be used to better understand the mechanisms of arrhythmia in ARVC patients.

It is important to recognize some key limitations of iPSCs. Primarily, myocytes derived from iPSCs have an immature phenotype especially in regard to structure and calcium regulation (Yang, Pabon, & Murry, 2014). However, a more mature phenotype can be achieved by promoting mature metabolism (Kim et al., 2013), plating cells on aligned nano-fibers (Vasireddi et al., 2018), and utilizing tissue constructs (Blazeski et al., 2019). Nonetheless, the immaturity of myocytes derived from iPSCs may be an advantage for ARVC or any disease with an early onset. Finally, cardiac myocytes derived from iPSCs contain a heterogeneous population of atrial, ventricular, and nodal like cells (Talkhabi, Aghdami, & Baharvand, 2016).

## 7 | SUMMARY

Human stem cells have played a critical role in elucidating mechanisms of heart disease. Such is the case for ARVC, an inherited cardiac muscle disorder that is associated with a high incidence of fatal ventricular arrhythmias especially during exercise and there is, currently, no cure. While fatty replacement, a hallmark of ARVC, was originally thought to be a congenital abnormality, it is now recognized to be a progressive disease process that can begin early in life. Genetic mouse models replicate the variable expressivity of ARVC observed in humans and they have provided fundamental insights to arrhythmia mechanisms. Yet, the complexity of the disorder is underscored by the molecular mechanisms associated with desmosome protein variants that interfere with Wnt signaling and how these might promote arrhythmogenesis. Importantly, while it is easy to explain the structural impact of fibro-fatty infiltrate on arrhythmogenesis, it has become increasingly apparent that nonmyocytes and associated metabolic and inflammatory mediators can have a significant impact as well. To gain a better understanding, myocytes and nonmyocytes derived from patient specific stem cells (iPSCs) may prove particularly useful because, in part, it is possible to study their relative importance that is specific to humans with ARVC. Thus far, findings emphasize the

importance of the fibro-fatty infiltrate. Finally, targeting the metabolic and inflammatory mediators associated with fibro-fatty infiltrate might lead to novel therapeutic options for mitigation of arrhythmias in ARVC patients.

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## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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