Forever Young: Induced Pluripotent Stem Cells as Models of Inherited Arrhythmias

Running title: Park et al.; IPS cells as arrhythmia models

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Mutations in the α subunit of the cardiac sodium channel, encoded by SCN5A, have been identified in a broad range of cardiac rhythm disorders that include long QT3 (LQT3), Brugada syndrome, progressive cardiac conduction disease, sick sinus syndrome, atrial fibrillation, and dilated cardiomyopathy. Since the identification of the first SCN5A mutation in long QT syndrome in 1995, more than 200 mutations have now been reported. Initially, it was assumed that each arrhythmic phenotype was attributable to a specific SCN5A mutation; however, "overlap syndromes", where single individuals exhibit clinical features of Brugada syndrome and LQT3, are now well characterized. The broad phenotypic range of SCN5A mutations underscores the importance of tight sodium channel regulation in maintaining normal rhythmicity.

The cardiac sodium channel is part of a macromolecular complex that includes the pore-forming α subunit and ancillary modulatory β-subunits, syntrophin, dystrophin, ankyrin, caveolin-3, Nedd4-like ubiquitin-protein ligases and calmodulin. This large complex brings together cytoskeletal proteins, signal transduction pathways, and cell adhesion molecules, allowing precise regulation of channel gating and kinetics, channel biosynthesis and degradation, and trafficking. Proper localization of Na1.5 at intercalated discs within caveolar domains is critical for rapid impulse propagation. The identification of mutations in caveolin-3, the main structural protein of caveolae within striated muscle, in long QT patients underscored the importance of structural proteins in modulating sodium channel activity. Co-expression of mutant caveolin-3 with wildtype, human Na1.5 in HEK-293 cells results in a 2- to 3-fold increase in late sodium current, mimicking a Na1.5 gain-of-function phenotype. Furthermore, mutations in the ankyrin-binding motif in Na1.5 (E1053K) identified in a patient with Brugada syndrome result in disrupted ankyrin-G binding and loss of Na1.5 surface expression in rat
cardiomyocytes. These results demonstrate the importance of the proper cellular environment when studying sodium channel physiology.

Characterization of sodium channel mutations using heterologous expression systems in non-excit able cells can produce inconsistent, and even confounding results. A striking example was reported by Roden and colleagues, who showed that $D1275N\text{SCN5A}$ expressed in knock-in mice demonstrated slowed conduction, heart block, atrial and ventricular arrhythmias, and dilated cardiomyopathy, faithfully phenocopying the human disease. While isolated ventricular myocytes from $D1275N$ mutant mice displayed reduced sodium channel abundance and reduced peak sodium current, the same mutation heterologously expressed in Chinese hamster ovary cells or tsA201, with or without the $\beta 1$ subunit, showed near-normal sodium channel function. Similarly, overexpression of the Ankyrin-G binding mutant, $E1053K\text{SCN5A}$, in HEK-293 cells showed normal membrane targeting, whereas expression of the mutant channel in ventricular myocytes abolished proper membrane trafficking.

Harnessing the power of induced pluripotent stem cells (iPSC) would obviate the need for heterologous expression systems, as potentially limitless supplies of personalized cardiomyocytes could be generated. This approach would, in theory, circumvent species-specific and inter-personal variations, allowing unprecedented resolution of channel behavior from patient-derived cells. For this reason, momentum has grown, both in bio-banking patient specimens and in generating cardiomyocytes from iPSCs (iPSC-CM) to recapitulate human channelopathies in a dish. However, tempering this enthusiasm for iPSC-CMs to model adult channelopathies is the well-described, phenotypic immaturity of iPSC-CMs. Electrophysiologically, cardiomyocytes derived from human embryonic stem cells (hESCs) and hiPSCs have significantly reduced upstroke velocities and have relatively depolarized resting
membrane potentials, mimicking fetal cardiomyocytes. Similarly, mouse iPSC-CMs exhibit delayed maturation, even after prolonged differentiation, when compared to mESC-derived cardiomyocytes and fetal myocytes, possibly due to epigenetic memory. Therefore, the feasibility of using iPSC-CMs in studying sodium channel physiology, in particular, has been questioned.

In this issue of *Circulation*, Mummery and colleagues specifically address the issue of whether cardiomyocytes derived from several pluripotent stem cell lines (mESC, miPSC, and hiPSC) can serve as a suitable model for studying complex sodium channel mutations. To that end, electrophysiological analyses were performed on derived cardiomyocytes bearing the murine equivalent of the human 1795insD SCN5A mutation, the first characterized overlap mutation exhibiting features of both Brugada syndrome and LQT3. Cardiomyocytes were generated from miPSCs derived from Scn5a1798insD/+ tail tip fibroblasts or MEFs and from mESCs used to create the Scn5a1798insD/+ mouse. Biophysical analysis demonstrated features of both loss-of-function (reduced sodium current density) and gain-of-function (larger persistent INa), mirroring the defects noted in primary Scn5a1798insD/+ adult cardiomyocytes. Corresponding changes in action potential recordings such as reduced upstroke velocity and prolonged action potential duration were noted in mutant myocytes. Furthermore, hiPSC-CMs generated from an affected individual recapitulated the biophysical abnormalities noted in mPSC-derived cardiomyocytes. Therefore, while the phenotypic immaturity of cardiomyocytes derived from mESCs and m/hiPSCs has served as a significant hurdle for regenerative applications, their tendency to remain “forever young” does not preclude their use in studying complex sodium channel mutations exhibiting both loss- and gain-of-function mutations.

The concept of using iPSC-CMs for studying sodium channel mutations has significant
implications. The ability to perform expression profiling between family members or ideally, identical twins who manifest significant phenotypic variability can help to identify critical genetic modifiers of disease severity. iPSC-CMs can also move us closer to personalized medicine for Brugada syndrome and LQT3, where drug regimens can be tested in vitro prior to being administered to patients. For example, mexilitine has been found to have a beneficial effect on shortening the QT interval in LQT3, but not in all patients. After a systematic biophysical characterization of mutant channels, Priori and colleagues concluded that a hyperpolarizing shift of inactivation may predict a positive response to mexilitine.\textsuperscript{14}

Furthermore, sodium channel blockade may be detrimental for patients with overlap syndromes. Therefore, the ability to screen patient’s iPSC-CM’s for response to drug therapy, while avoiding untoward effects may move us closer to tailored therapy for inherited channelopathies.

Another potential area of growth for iPSC-CMs will likely be in high throughput/high content screens to rapidly identify novel si-RNAs, small molecules, or drugs that can correct trafficking defects, alter sodium channel activity, or enhance translational readthrough of nonsense mutations. Nonsense mutations, which introduce a premature termination codon (PTC) in the open reading frame of an allele, have been identified in \textit{SCN5A}. Enhancing translational readthrough is an emerging technology that enables ribosomes to bypass PTCs, allowing full-length proteins to be made.\textsuperscript{15} Two strategies to improve readthrough have been reported in an \textit{SCN5A} nonsense mutation (\textit{W822X}): 1) reduction of translational fidelity using aminoglycosides and 2) decreased translation termination efficiency using siRNA targeting the eukaryotic release factor, eRF3a.\textsuperscript{15} Either approach restored I\textsubscript{Na} to \(~30\%\) from \(<3\%\) of wildtype levels by increasing full-length protein expression. Translational readthrough-corrected channels behaved similarly to wildtype channels in regards to kinetics and voltage dependency. Furthermore, there
was no evidence of abnormal readthrough of natural stop codons. A major limitation of this work was the use of heterologous expression of *W822X SCN5A* in HEK-293 cells.\textsuperscript{15}

Recapitulation of this work in iPSC-CMs is warranted and would provide a more accurate readout of enhanced readthrough on full-length sodium channel expression and function in the endogenous cardiomyocyte.

**Conflict of Interest Disclosures:** None

**References:**


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