Voltage-Gated Channel Alpha Subunit 5 (SCN5A), the main alpha-subunit of the cardiac sodium channel isoform. Our previous work demonstrated that SCN10Ashort, a small fragment comprising of the C-terminus of SCN10A, increased sodium current when co-expressed with SCN5A. In the present study we therefore explored SCN10Ashort as a novel gene therapy target.

**Objective:** To validate SCN10Ashort as a gene therapy target to increase the cardiac sodium current.

**Methods:** HEK cells with stable SCN5A expression were transfected with GFP or SCN10Ashort-FLAG-YFP and fixed for immunocytochemistry. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were transduced with GFP and SCN10Ashort-YFP lentiviral vectors. Sodium current and APs were measured using patch-clamp methodology. For in vivo studies, GFP and SCN10Ashort-YFP were packaged in adeno-associated viral (AAV) vectors and these vectors were validated by immunocytochemistry. Next, AAV vectors were injected into the left ventricle free wall of adult mice. Two weeks after injection, mice were sacrificed and cardiomyocytes were isolated for patch-clamping.

**Results:** Immunocytochemistry on HEK cells showed membrane expression of SCN10Ashort, co-localized with SCN5A. In hiPSC-CMs, overexpression of SCN10Ashort leads to a significant increase in AP upstroke velocity (195.5 ± 29.1 vs 113 ± 12.4, p < 0.01), compared to the GFP group. Other AP parameters were not significantly affected. In vivo studies confirmed that overexpression of SCN10Ashort increases AP upstroke velocity (302.4 ± 17.5 vs 214.1 ± 8.8, p < 0.01 versus GFP group). AP duration at 20% repolarization (APD_{20}) was reduced (2.8 ± 0.4 vs 1.3 ± 0.1, p < 0.05), but APD_{50} and APD_{90} were unchanged.

**Conclusion:** The robust increase in sodium current after in vivo gene transfer with SCN10Ashort carries substantial promise for novel gene therapy applications for inherited sodium channelopathies and acquired pacemaker and conduction system disorders.

**BS-514-02**

**TRANSIENT BIOLOGICAL PACING BASED ON AAV6-HCN2/SKM1 GENE TRANSFER IN PIGS WITH COMPLETE HEART BLOCK**

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**Background:** In an effort to develop hardware-free pacemakers, we and others have engaged into the development of gene therapy-based biological pacemakers by means of ion channel overexpression. Here we report our progress with regard to adeno associated virus (AAV) vectors-mediated delivery of HCN2/SKm1 to generate long-term biological pacing.

**Objective:** To study the long-term efficacy of biological pacing based on AAV-mediated gene transfer of HCN2/SKm1.

**Methods:** We tested in vitro AAV-mediated overexpression of HCN2 and SKM1 on transduced neonatal rat ventricular myocytes (NRVMs). In vivo gene transfer was further validated after injecting 1E11 GC of AAV6-cTNT-HCN2 or AAV6-CMV-SKM1 into the left ventricle free wall of adult mice. Functional biological pacemaker studies were performed in pigs with radiofrequency ablation-induced complete AV block (CABV). Four weeks after the ablation, we proceeded with three groups: non-injected, saline, and AAV6-HCN2/SKm1 (5E12 GC per vector). All animals were then observed for another four weeks to evaluate in vivo biological pacemaker performance.

**Results:** AAV6-HCN2 and AAV6-SKM1 showed both robust expression in vitro (NRVMs) and in vivo (mice) (Figure 1B and 1C). We thus proceeded with in vivo testing in CAVB pigs. Within the first two weeks of gene delivery, maximal beating rate increased in the HCN2/SKm1-transduced animals (Figure 2B) and mean heart rate modestly trended up after one week after injection, although this trend was not sustained (Figure 2A). This increase in beating rates was accompanied by an abrupt reduction of electronically paced beats in HCN2/SKm1 animals showing dependency on backup pacing of less than 50% - one week after of transgene delivery (Figure 2C). Finally, we evaluated corrected ventricular recovery times after overdrive suppression pacing which indicated enhanced intrinsic pacemaker activity in HCN2/SKm1-injected animals (Figure 2D).

**Conclusion:** AAV-mediated gene transfer results in robust long-term overexpression of HCN2 and SKM1 in mouse myocardium. AAV-HCN2/SKm1 gene transfer in CAVB pigs resulted in transient biological pacemaker activity, potentially stemming from insufficient in vivo transduction and/or immunological interference.

**Figure 1.** AAV6-cTNT-HCN2 or AAV6-CMV-SKM1 (via two overlapping vectors) transduced cardiomyocytes in vitro and in vivo. (A) Schematic representation of AAV6-cTNT-HCN2 and AAV6-CMV-SKM1 vectors. SKM1 vector share an overlapping region which induces recombination. (B) HCN2 and (C) SKM1 expression was detected by immunohistochemistry in NRVM and mouse left ventricle free wall. Untransduced NRVM (UT) and saline-injected animals were used as negative control.

**Figure 2.** HCN2/SKM1 generates a transient biological pacemaker phenotype in CAVB pigs. (A) Mean and (B) maximal basal rates collected from saline, untransduced and transduced animals over the course of eight weeks. Data are means ± SEM (n = 3), saline and n = 2, transduced animals n = 3, (D) echocardiographic parameter usage eight weeks after creation of a heart block (n = 3, saline = 3, HCN2/SKM1 = 2). (B) Measurement of corrected ventricular recovery time after an overdrive suppression protocol four weeks post intracoronary injections in sham animals and HCN2/SKm1-treated animals. Data are means ± SEM (n = 3, HCN2/SKM1 animals n = 3).

**BS-514-03**

**CRISPR GENE MODULATION IN PRE-DIFFERENTIATED HIPSC-CMS COMBINED WITH ALL-OPTICAL ELECTROPHYSIOLOGY**

Julie Han BA and Emilia Entcheva PhD

**Background:** Current models for cardiotoxicity screening are limited to only evaluate hERG inhibition for related prediction of QT prolongation and torsadogenic potential. More comprehensive in vitro cardiotoxicity assays are being proposed using human induced pluripotent stem cell-derived
cardiomyocytes (iPSC-CMs), aided with experimental and computational approaches.

Objective: We tested the potential of CRISPRi modulation in pre-differentiated iPSC-CMs combining all-optical cardiac electrophysiology and molecular analysis to characterize functional phenotypes of key ion channels in the human heart.

Methods: We designed single guide RNAs (sgRNAs) targeting KCNH2, KCNJ2, and GJA1. The sgRNAs were cloned into a lentiviral vector with eGFP reporter. A dox-inducible dCas9-KRAB was inserted into the AAVS1 safe harbor site of pre-differentiated hiPSC-CMs and transduced with lentivirus carrying the sgRNAs. To deploy all-optical electrophysiology, cells were transduced with an adenoviral vector containing ChR2-eYFP (for optical stimulation) and co-labeled with spectrally-compatible voltage and calcium sensors to obtain functional measurements upon 5 days of dox induction.

Results: A sgRNA targeting KCNH2 resulted in about 40% knockdown of KCNH2 mRNA and significant but mildly prolonged action potential duration, e.g. spontaneous APD90 (+12%, p < 0.05). A sgRNA targeting KCNJ2 caused about 60% downregulation of mRNA levels and exhibited minimal changes in spontaneous beat frequency in high density cell preparations (+7%, n.s.) but more pronounced effects in reduced density cell preparations (+30%, p < 0.01). Additionally, sgRNA targeting GJA1 exhibited about 40% knockdown of mRNA and resulted in significant slowing of conduction velocity (-19%, p < 0.01) at 1 Hz pacing conditions.

Conclusion: Knockdown of key cardiac ion channels in our system yielded mild but specific functional changes. Using this platform for CRISPRi mediated knockdown of disease-associated genes in pre-differentiated cardiomyocytes can improve the assessment of gene function in cellular cardiac electrophysiology. In combination with sgRNA libraries and/or with CRISPRa mediated gene activation, it can allow a more comprehensive evaluation of the mechanisms controlling cardiac electromechanics.

BS-514-04

ADENOVIRUS INCREASES ARRHYTHMIA SUSCEPTIBILITY DURING ACUTE CARDIAC INFECTION

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Background: Myocarditis underlies 42% of sudden cardiac death in young adults, yet viral arrhythmogenic mechanisms remain elusive. Adenovirus is a leading cause but species-specificity has limited disease modeling in mice. Gap junctions, composed primarily of connexin43 (Cx43), enable electrical impulse propagation in the heart. Changes in Cx43 expression, localization, and/or function cause arrhythmias. Gap junctions propagate innate and adaptive antiviral responses, and our prior work has demonstrated that Cx43 expression and function are reduced during human adenoviral infection.

Objective: Utilize cardiotropic Mouse Adenovirus Type-3 (MAdV-3) to investigate how acute viral infection generates an arrhythmogenic substrate.

Methods: Adult mice were inoculated with MAdV-3 and viral genomes quantified by qPCR for organ tropism along with histopathology after 7 days. Cardiac function was measured by echo- and electrocardiography, and optical mapping. Changes to ion channel expression and localization were quantified using RT-qPCR, western blotting, confocal, and super resolution microscopy of infected tissue and isolated adult ventricular cardiomyocytes (ACMs), which also underwent patch clamping.

Results: Viral genomes were specifically enriched in heart tissue and no cardiomyopathy was detected, just as in human acute myocarditis. Reductions in cardiac ion channel and connexin mRNA levels occurred in infected hearts. Cx43 was phosphorylated at residues known to reduce function and decreased conduction velocity was found in infected hearts by optical mapping. Prolonged action potential duration was detected with impaired K+ currents in infected ACMs by patch clamping. Microscopy of infected cardiac tissue and ACMs revealed alterations in gap junction/scaffolding protein complexing at the cell-cell junction. In human iPSC-derived cardiomyocytes, Cx43 phosphorylation was increased and cellular uncoupling detected by optical mapping during human adenovirus infection.

Conclusion: Reduced cellular coupling and ion channel function during adenoviral infection generates an arrhythmogenic substrate prior to an appreciable immune response or cardiomyopathy development, highlighting contributions of active infection to electrical disturbances prior to inflammation.

ABSTRACT CA-531:

Insights and Innovations in Ventricular Arrhythmia Ablation

Saturday, April 30, 2022
10:30 AM - 11:30 AM

CA-531-01

FAT INFECTION CONFERS PROPENSITY FOR VENTRICULAR TACHYCARDIA IN THE POST-INFARCT SUBSTRATE

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Background: Infiltrating adipose tissue (inFAT) is present in the post-infarct substrate, but its role in ventricular tachycardia (VT) arrhythmogenesis is not well-established.

Objective: To investigate the role of post-infarct inFAT in VT propensity.

Methods: 24 post-infarct patients who underwent VT ablation were prospectively enrolled across two centers. For each patient, contrast-enhanced computed tomography (CE-CT), late gadolinium-enhanced magnetic resonance imaging (LGE-MRI), and substrate mapping during sinus rhythm were acquired. Voltage amplitude, deceleration zones (DZ) which represent regions of activation slowing, and substrate-based ablation locations were compared with the inFAT and scar distributions. To glean deeper insights into arrhythmogenic propensity, novel hybrid digital heart twins representing the patient-specific inFAT from CT and scar from MRI were reconstructed. VT circuits were then induced, assessed, and compared to mapping data (Fig.A).

Results: Combined inFAT and scar had lower voltage and exhibited stronger correlations with voltage amplitude than that of scar alone (0.76 ± 0.56 vs. 1.19 ± 0.47 mV, p < 0.005; r = -0.54 vs. -0.45). InFAT and scar exhibited greater isochronal crowding than scar alone (32/2 vs. 21/1 isochrones, p < 0.005). Most DZs consisted of combined inFAT and scar (71.1%) rather than scar alone. The amount of ablated inFAT was strongly correlated with ablated scar (r = 0.73, p < 0.05). In the digital hearts, 140 VTs were ablated. The total amount of inFAT, but not scar, was significantly associated with the number of VTs induced (p < 0.05). For most VT circuits, the critical isthmus was comprised of both inFAT and scar (110/140 VTs) (Fig.B). In a