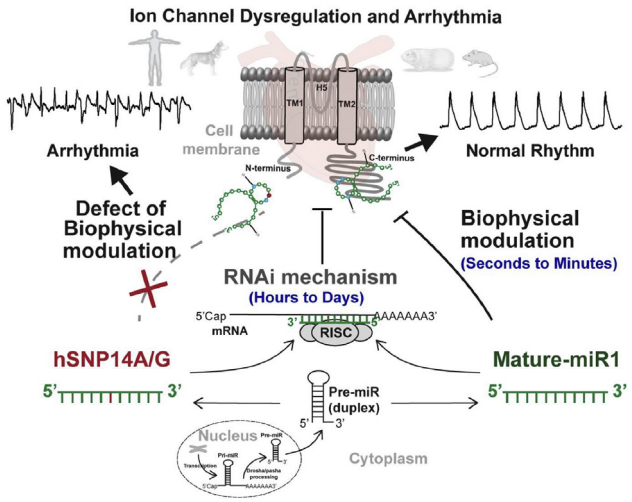

B-AB18-02

KIR2.1 CHANNELS IN A NOVEL SARCOPLASMIC RETICULUM MICRODOMAIN CONTROL INTRACELLULAR CA^{2+} DYNAMICS

Alvaro Macias PhD, Andrés González-Guerra, Ana I. Moreno-Manuel, Francisco M. Cruz, Nieves García-Quintáns, Lilian K. Gutiérrez, Marta Roche-Molina, Francisco Bermúdez-Jiménez, María L. Vera-Pedrosa, Isabel Martínez-Carrascoso, Juan A. Bernal and Jose Jalife MD, FHRS

Background: The strong inward rectifier K^+ channel, Kir2.1, is known to localize at the sarcolemma to control the resting potential and the final phase of ventricular repolarization. K^+ channels have been suggested to contribute countercurrent to calcium flux across the sarcoplasmic reticulum (SR) membrane, but their identity and function remain controversial.
Objective: To test whether a fraction of Kir2.1 channels that cluster within a novel SR membrane microdomain function to provide essential countercurrent to balance Ca^{2+} reuptake, helping to control intracellular calcium dynamics and excitation-contraction coupling.
Methods: Using confocal microscopy we analyzed the ultrastructure of mouse and rat skeletal muscle slices, cardiomyocytes, and isolated mouse cardiac SR vesicles. Immunolocalization of target proteins was analyzed in intact and detubulated cardiomyocytes treated with formamide by immunofluorescence and biochemically by western-blotting after membrane fractionation. Functional assays included patch-clamping and calcium transient dynamics.
Results: Cardiomyocytes and skeletal muscle slices revealed two distinct microdomain bands of Kir2.1 immunostaining, one colocalizing with NaV1.5 near the Z disk, the other colocalizing with Ankyrin-B in the M line. The latter is a previously unknown Kir2.1 channel microdomain localized at the SR membrane. Its ionic current was sensitive to spermine and caffeine, and modified by asymmetrical potassium concentrations. Finally, chloroquine-mediated inhibition of the SR Kir2.1 current resulted in a larger but slower calcium SR reuptake. Hence, we revealed a previously unknown physiological role for Kir2.1 channels at the SR membrane in the control of intracellular Ca^{2+} dynamics, conducting K^+ as a potential countercurrent ion to calcium reuptake.
Conclusion: Altogether, the data provide original structural and functional demonstration of a major K^+ channel localizing at the SR and contributing to the control of intracellular calcium homeostasis. Aberrant Kir2.1 localization at the SR could underly cardiac arrhythmogenesis and periodic skeletal muscle paralysis in several inheritable ion channel diseases.

B-AB18-03

SARS-COV-2 DIRECT CARDIAC DAMAGE THROUGH SPIKE-MEDIATED CARDIOMYOCYTE FUSION MAY CONTRIBUTE TO INCREASED ARRHYTHMIC RISK IN COVID-19

Daniel J. Clemens BS, Dan Ye MD, Changsung John Kim PhD, David R. Pease BS, Chanakha K. Navaratnarajah PhD, Alison Barkhymer BS, Timothy J. Nelson MD, PhD, Roberto Cattaneo PhD, Jay W. Schneider MD, PhD and Michael John Ackerman MD, PhD

Background: SARS-CoV-2-mediated COVID-19 can affect the heart and cause sudden cardiac death (SCD). Multiple factors contribute to this increased risk of potentially fatal arrhythmias including thrombosis, exaggerated immune response, and treatment with QT-prolonging drugs. However, the intrinsic arrhythmic potential of direct SARS-CoV-2 infection of the heart is unknown.
Objective: To assess the cellular and electrophysiological effects of direct SARS-CoV-2 infection of the heart using induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).
Methods: iPSC-CMs were either infected with SARS-CoV-2 or transfected with recombinant SARS-CoV-2 spike protein (S). Viral replication and iPSC-CM morphology were visualized by electron and confocal microscopy respectively. Action potential duration (APD) and cellular arrhythmias were measured by whole cell patch-clamp. Ca^{2+} handling was assessed by live cell imaging using the Fluo-4 Ca^{2+} indicator.
Results: Following infection with SARS-CoV-2, iPSC-CMs expressed viral proteins and displayed efficient virus production. Infected cells produced multinucleated giant cells called syncytia resulting from cell-cell fusion. Interestingly, S-transfected iPSC-CMs also produced syncytia indicating fusion is S-mediated. SARS-CoV-2 S-induced syncytia displayed increased cellular capacitance (75 ± 7 pF, $n=10$ vs. 26 ± 3 pF, $n=10$; $P<0.0001$) consistent with increased cell size. The APD90 was prolonged significantly from 419 ± 26 ms ($n=10$) in untransfected iPSC-CMs to 590 ± 67 ms ($n=10$; $P<0.05$) in S-transfected iPSC-CMs. Additionally, these syncytia displayed delayed afterdepolarizations and erratic beating frequency. Ca^{2+} imaging revealed Ca^{2+} handling abnormalities in S-induced syncytia including Ca^{2+} sparks and, most notably, large “tsunami”-like waves. After either treating with a furin protease inhibitor or mutating the S furin cleavage site, cell-cell fusion was no longer evident and Ca^{2+} handling returned to normal.
Conclusion: The SARS-CoV-2 spike protein can directly produce cellular damage and electrophysiological dysfunction in infected cardiac cells which may confer the intrinsic, mechanistic susceptibility for the increased risk of SCD observed in COVID-19.

B-AB18-04

SGK1 DEFICIENCY IS PROTECTIVE IN A MODEL OF SEPSIS-RELATED ATRIAL FIBRILLATION

Aneesh Bapat MD, Maximilian J. Schloss, Matthias Nahrendorf, Patrick T. Ellinor MD, PhD, David J. Milan MD and Saumya Das MD, PhD

Background: Sepsis is associated with new onset atrial fibrillation (AF), and AF in septic patients is associated with worse outcomes. Serum glucocorticoid kinase 1 (SGK1), a serine/threonine kinase in the PI-3 kinase pathway, is involved in cardiac