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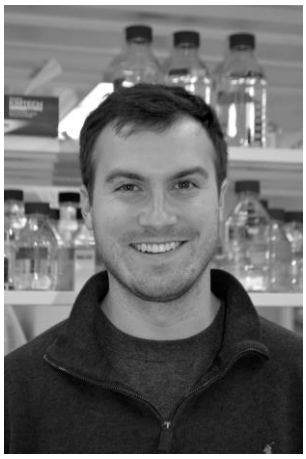
External Seminar



Speaker

Jonathan Lambert, BS
Center for Translational Medicine
Lewis Katz School of Medicine at Temple University
Philadelphia

Place: **Analysezentrum 3, 2. OG, Room 02.332**
Date: **Friday, June 7th**
Time: **1.00 pm**

MCUB regulates the molecular composition of the mitochondrial calcium uniporter channel to limit mitochondrial calcium overload during cardiac stress

The mitochondrial calcium uniporter channel (mtCU) is a multi-subunit complex that resides in the inner mitochondrial membrane and is required for mitochondrial Ca^{2+} ($_{m}Ca^{2+}$) uptake. Mitochondrial Calcium Uniporter B (MCUB, *CCDC109B* gene), a recently identified paralog of *MCU*, is reported to negatively regulate $_{m}Ca^{2+}$ uptake; however, its precise regulation of uniporter function and contribution to cardiac physiology remain unresolved. Size exclusion chromatography of mitochondria isolated from ventricular tissue revealed MCUB was undetectable in the high-molecular weight (MW) fraction of sham animals (~700kD, size of functional mtCU), but 24 hours following myocardial ischemia-reperfusion injury (IR) MCUB was clearly observed in the high-MW fraction. To investigate how *MCUB* contributes to mtCU regulation we created a *MCUB*$^{-/-}$ cell line using CRISPR-Cas9n. *MCUB* deletion increased histamine-mediated [$_{m}Ca^{2+}$] transient amplitude by ~50% vs. WT controls (mito-R-GECO1). Further, *MCUB* deletion increased mtCU capacitance (patch-clamp) and rate of [$_{m}Ca^{2+}$] uptake. FPLC fractionation of the mtCU revealed that loss of MCUB increased MCU incorporation into the high-MW complex suggesting stoichiometric replacement and overall increase in functional mtCU complexes. Next, we generated a cardiac-specific, tamoxifen-inducible MCUB mouse model (CAG-CAT-MCUB x MCM; MCUB-Tg) to examine how the MCUB/MCU ratio regulates mtCU function and cardiac physiology. MCUB-Tg mice were infected with AAV9-mitycam ($_{m}Ca^{2+}$ reporter) and adult cardiomyocytes were isolated to record [$_{m}Ca^{2+}$] transients during pacing. Increasing the MCUB/MCU ratio decreased [$_{m}Ca^{2+}$] peak amplitude by ~30% and significantly reduced the [$_{m}Ca^{2+}$] uptake rate. FPLC assessment revealed MCUB was undetectable in the high-MW fraction of Cre controls, but enriched in MCUB-Tg hearts. MCUB incorporation into the mtCU decreased the overall size of the uniporter and reduced the presence of channel gatekeepers, MICU1/2. Immunoprecipitations suggest that MCUB directly interacts with MCU but does not bind MICU1/2. These results suggest that MCUB replaces MCU in the mtCU and thereby modulates the association of MICU1/2 to regulate channel gating. Cardiomyocytes isolated from MCUB-Tg hearts displayed decreased maximal respiration and reserve capacity,

which correlated with a severe impairment in contractile reserve (LV invasive hemodynamics). MCUB-Tg cardiac mitochondria were resistant to Ca^{2+}-induced mitochondrial swelling suggesting MCUB limits mitochondrial permeability transition. Further, MCUB-Tg mice subjected to *in vivo* myocardial IR revealed a ~50% decrease in infarct size per area-at-risk. These data suggest that MCUB regulation of the mtCU is an endogenous compensatory mechanism to decrease $_{m}Ca^{2+}$ overload during ischemic injury, but this expression is maladaptive to cardiac energetic responsiveness and contractility.

Host: **Prof. Dr. med. Johannes Backs**
Director of the Department Molecular Cardiology and Epigenetics
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