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Abstracts

ST01

Mitochondrial dysfunction in M2 macrophages differentiated from human non-classical monocytes is linked to foam cell formation

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Intermediate (CD14+CD16+) and non-classical (CD14dimCD16+), but not classical (CD14+CD16-) monocytes, are elevated in patients with cardiovascular disease. It is unknown how these monocytes handle lipids once they become macropahges.
We aimed to determine the lipid-handling and metabolic phenotype of macrophages derived from human monocyte subsets and to examine this in a mouse model of atherosclerosis.
We found that M2 macrophages from all human monocyte subsets express lipid uptake receptor genes, and took up oxLDL, where M1 macrophages did not. Following oxLDL loading, M2 macrophages from classical and intermediate monocytes displayed upregulated ABCA1 gene expression and also increased cholesterol efflux to apoA-I. This was not observed in M2 macrophages derived from non-classical monocytes, suggesting impaired cholesterol efflux. Moreover, glycolytic capacity was increased in these cells while mitochondrial activity decreased after oxLDL loading, suggesting potential dysfunction of the mitochondria. This metabolic phenotype was also confirmed ex vivo in aortic plaque M2 macrophages from Apoe-/- mice. Mitochondrial dysfunction was later discovered to be due to a robust increase in mitochondrial reactive oxygen species (mtROS) production in M2 macrophages from human non-classical monocytes and aortic arch of Apoe-/- mice. When mitochondrial activity of oxLDL-loaded M2 macrophages from non-classical monocytes were improved by reducing mtROS with MitoQ, cholesterol efflux capacity was restored. Treatment of Apoe-/- mice with MitoQ also reduced atherosclerosis. We suggest that non-classical monocyte derived M2 macrophages are involved in foam cell formation, which is caused by mitochondrial dysfunction. Restoring mitochondrial function restores cholesterol efflux potential and could aid in atherosclerotic lesion regression.

ST02

Desmosomal degradation as an underlying cause for arrhythmogenic cardiomyopathy

Hoyee (Jenny) Tsui[1], Sebastiaan van Kampen[1], Su-Ji Han[1], Petra van der Kraak[2], Aryan Vink[2], Xiaoke Yin[3], Manuel Mayr[3], Simona Casini[4], Alexander Bossu[5], Gerard Marchal[4], Jantine Monshouwer-Kloots[1], Joep Eding[1], Danielle Versteeg[1], Toon van Veen[5], Carol Ann Remme[4], Eva van Rooij[1]

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Arrhythmogenic cardiomyopathy (ACM) is an inherited progressive cardiomyopathy with a prevalence of 1:2000 to 1:5000. Approximately 50% of ACM cases are caused by mutations in desmosomal genes, with the majority in the plakophilin 2 (PKP2) gene. While much is known about the disease genetic origin, the disease driving mechanisms remain undefined.

Here, CRISPR/CAS9 was utilized to generate the first Pkp2 knock-in mouse model bearing a human pathogenic mutation PKP2c.2013delC (mouse equivalent Pkp2c.1755delA, Pkp2-c.1755delA/WT). These mutant mice exhibited Pkp2 haploinsufficiency compared with controls. Proteomics analysis on cardiac tissue lysates from young mice revealed a striking molecular phenotype where all major desmosomal, adherens junction (AJ) and ACM-related proteins were decreased in mutant mice; validated through immunoblots. Furthermore, 12-month-old Pkp2-c.1755delA/WT mice demonstrated desmosomal protein disarray in regions of pathological fibrotic remodeling, increased levels of fibrosis and signs of diastolic dysfunction compared to controls. Intriguingly, the aforementioned characteristics of reduced desmosomal and AJ proteins, desmosomal protein disarray in pathological fibrotic regions and diastolic dysfunction are ACM features that recapitulate those illustrated in explanted ACM patients. Led by proteomics data showing involvement of the ubiquitin-proteasome system (UPS) and in the absence of mRNA changes, we successfully rescued desmosomal protein levels in Pkp2-c.1755delA/WT mice using a UPS inhibitor.

In conclusion, our data demonstrate the role of UPS directed desmosomal protein degradation as an underlying cause for ACM; these molecular phenotypes precede functional deficit. Defining the underlying pathway(s) responsible for protein degradation could aid in therapeutic strategies for desmosomal stabilization which would be beneficial for ACM patients.

ST03

Optimization of measurement of mitochondrial electron transport activity in frozen heart samples.

Gloria Benavides[1], Victor Darley-usmar[1]
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It is well known that the heart mitochondria are critical to meet the energetic and metabolic requirements of the heart and are tightly regulated. Abnormalities in heart mitochondrial function, due to genetic, mitochondrial toxins, aging or defects in mitochondrial quality control contribute to cardiac disease. It has been challenging to relate mitochondrial function to complex pathological processes such diabetes in the heart due to the availability of material and the sensitivity of the assay methods. We have recently established protocols using as little as 10 mg of heart tissue to measure mitochondrial electron transport activity in frozen samples without the need to isolate mitochondria. This important as it avoids selection of mitochondrial sub-populations during the preparation and greatly improves throughput and sensitivity. We will show how we optimized these methods to determine the activities of mitochondrial electron transport in frozen heart tissues. The establishment of such an approach will significantly impact translational studies using animal models or human samples by allowing measurement of mitochondrial function in stored samples and from human tissue repositories.

ST04

Ceramides Alter Glucose Metabolism and Drive Diastolic Dysfunction by Decreasing Isocitrate Dehydrogenase 2 Expression

Sean Tatum[1], Kyle Dunlap[1], Ahmad Cluntun[1], Alan J. Mashchek[1], Gregory Ducker[1], Stavros Drakos[1], Jared Rutter[1], Scott Summers[1], William Holland[1]
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Heart failure with preserved ejection fraction (HFpEF) is now the most common form of heart failure in the United States. The rapid rise in HFpEF incidence tightly correlates with rises in obesity and diabetes. Toxic sphingolipids like ceramides accumulate in obesity, linking over-nutrition to insulin resistance and other metabolic diseases like heart failure and are now used as clinical biomarkers of adverse cardiac events. Previously, our lab has shown that whole-body deletion of Degs1, the gene which encodes for the enzyme responsible for synthesizing ceramides from dihydroceramides, is sufficient to prevent insulin resistance, diastolic dysfunction and cardiac hypertrophy while preventing diabetes in leptin-deficient mice. Building on these data, we hypothesize that ceramide accumulation in the cardiomyocyte is requisite for the onset and progression of cardiac hypertrophy and HFpEF. To test our hypothesis, we generated an inducible heart-specific deletion of Degs1 and induced HFpEF by leptin deletion or challenging adult mice with L-NAME and high-fat diet for 10 weeks. Animals lacking Degs1 were protected from cardiac remodeling (hypertrophy and fibrosis), diastolic dysfunction and exercise intolerance. Mechanistically in both mice and H9c2 cells, we find that ceramide causes phosphorylation and inhibition of yes-associated-protein, subsequently decreasing transcription and translation of its target gene, isocitrate dehydrogenase-2, leading TCA cycle perturbances and increased cataplerosis. Our findings suggest that Degs1 may provide a novel therapeutic approach for patients with diabetes and HFpEF by preventing mitochondrial dysfunction, cardiac hypertrophy, interstitial fibrosis and diastolic dysfunction that cardiac ceramides trigger.

ST05

Live-cell imaging of mitochondrial redox stress in a transgenic mouse model of spontaneous atrial fibrillation

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In cardiomyocytes, Na+ influx via the sodium channel NaV1.5 is essential for the activation of excitation-contraction coupling. However, the human NaV1.5-F1759A gain-of-function mutation has been associated with the pathogenesis of atrial fibrillation (AF). We hypothesized that intracellular Na+ overload causes increased Na+/Ca2+ exchange both in the plasma membrane and inner mitochondrial membrane, which may together perturb intracellular Ca2+ signalling and mitochondrial function, ultimately leading to increased mitochondrial production of reactive oxygen species (ROS). To address this hypothesis, we studied the recently established double-transgenic (dTG) mouse model FLAG-NaV1.5-F1759A, expressing the transgenes under control of the cardiac specific αMHC promotor and backcrossed in the mitochondrially redox-competent C57Bl/6N background. Kaplan-Meier survival analysis of 47 wild type control and 46 dTG animals showed an overall mortality of 60% after 12 weeks, which was pronounced in male compared to female mice. During 10-minute 6-lead ECG recordings, spontaneous episodes of intermittent AF were confirmed in all dTG animals. Transthoracic echocardiography measurements demonstrated significant structural and functional changes of the atria in 8 weeks old mice, e.g. increased left atrial inner diameters (LA ID: 2.54 ± 0.12 mm in dTG vs. 1.70 ± 0.04 mm in WT, $p < 0.0001$) and depressed left atrial fractional shortening (LA FS: 3.49 ± 0.50 % in dTG vs. 17.19 ± 1.12 % in WT, $p < 0.0001$). In addition, we identified a moderately decreased left-ventricular systolic function, which is in line with an arrhythmia-induced cardiomyopathy in dTG mice. Confocal bright-field imaging of isolated atrial and ventricular myocytes showed an increased cellular size and length that confirmed atrial and not ventricular hypertrophic remodelling. Confocal live-cell membrane imaging revealed changes of the transverse-axial tubule network with significantly decreased transverse vs. increased axial tubule components, suggesting subcellular excitation-contraction coupling defects. For live-cell mitochondrial ROS imaging, we crossed the AF mouse model FLAG-NaV1.5-F1759A with redox biosensor mice expressing mitochondrial matrix-targeted roGFP to measure the glutathione redox potential EGSH. Interestingly, atrial myocytes of triple transgenic (TTG) biosensor mice showed a significantly decreased glutathione redox potential EGSH (EGSH: -296.30 ± 2.0mV in TTG vs. -284.90 ± 1.5 mV in WT, $p < 0.0001$). Taken together, these findings are consistent with the hypothesis that intracellular Na+ overload leads to mitochondrial dysregulation and increased oxidative stress, which may induce maladaptive atrial remodelling and perpetuates atrial fibrillation.

ST06

BACE1-AS lncRNA role in shared disease mechanisms between heart failure and Alzheimer's disease

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-Background: BACE1-antisense RNA (BACE1-AS) is a lncRNA antisense to the Beta-Secretase-1 (BACE1) gene encoding a key enzyme in the production of the β-amyloid peptide, associated to Alzheimer's disease (AD). In a previous study, we showed that the BACE1-AS/BACE1 axis is activated in heart failure patients, leading to β-amyloid accumulation in failing hearts. Accordingly, BACE1-AS expression in different cultured cardiac cell types induced the expression of BACE1 and β-amyloid that, in turn, triggered apoptosis. The mechanisms underlying BACE1-AS action are not fully elucidated yet. Indeed, current models based on miRNA "sponging" or "masking" inducing BACE1 post-transcriptional stabilization may not recapitulate all BACE1-AS functions.
-Methods and results: Pull-down experiments followed by RNA-Seq identified RNAs binding BACE1-AS in AC16 cardiomyocytes. These transcripts were enriched in RNAs mapping to genomic enhancer regions that were also hypo-methylated in AD brains. Next, the levels of genes under the control of a subset of these enhancers was assayed upon BACE1-AS overexpression in cardiomyocytes. It was found that their expression was induced, supporting the functional interaction between BACE1-AS and the identified enhancer loci.
To gain further insight on BACE1-AS function in cardiomyocytes, accessible chromatin sites induced by BACE1-AS overexpression were assayed by ATAC-Seq. Interestingly, also the regions identified by this approach were enriched in hypo-methylated enhancers in AD brains. One of them was the locus encompassing RNF214/BACE1/BACE1-AS mapping to active enhancer marks, indicating that BACE1-AS regulates transcriptionally the expression of BACE1.
-Conclusion: Collectively, these data suggest BACE1-AS as a node of shared disease-mechanisms between heart failure and AD.

ST07

Preserved cardiac performance and adrenergic response in a rabbit model with decreased ryanodine receptor 2 expression

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Ryanodine receptor 2 (RyR2) is an ion channel in the heart responsible for releasing into the cytosol most of the Ca2+ required for contraction. Proper regulation of RyR2 is critical for cardiac function, as highlighted by evidence showing that dysregulation of the channel leads to cardiac arrhythmias and sudden death. Lower RyR2 expression is also observed in some forms of heart failure; however, there is limited information on the impact of this change on excitation-contraction (e-c) coupling, Ca2+-dependent arrhythmias, and cardiac performance. We used a constitutive knock-out of RyR2 in rabbits (RyR2-KO) to assess the extent to which a stable decrease in RyR2 expression modulates Ca2+ handling in the heart.

Homozygous knock-out of RyR2 in rabbits is lethal. Remarkably, heterozygotes (KO+/-) show ~50% loss of RyR2 protein without developing an overt phenotype at the intact animal and whole heart levels. Instead, we found that KO+/- myocytes show (1) remodeling of RyR2 clusters, favoring smaller groups in which channels are more densely arranged; (2) lower Ca2+ spark frequency and amplitude; (3) slower rate of Ca2+ release and mild but significant desynchronization of the Ca2+ transient; and (4) a significant increase in RyR2 channel activity, likely due to post-translational regulation of the remaining RyR2 proteins.

Our data show that RyR2 deficiency, although remarkable at the molecular and subcellular level, has only a modest impact on global Ca2+ release and is fully compensated at the whole-heart level. This highlights the redundancy of RyR2 protein expression and the plasticity of the e-c coupling apparatus.

ST08

Molecular and functional characterization of a novel mutation in the cardiac ryanodine receptor (RyR2) associated with Long QT Syndrome

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Background: Long QT syndrome is characterized by prolongation of the QT interval in the ECG, syncope and sudden death. Mutations in genes encoding sarcolemmal ion channels and interacting proteins account for approximately 80% of all cases; however, the genetic links of the remaining 20% cases are unknown. Studies of LQTS patients negative for the most common gene mutations have recently revealed novel variants in the cardiac ryanodine receptor (RyR2), the Ca2+ release channel of sarcoplasmic reticulum that provides the majority of Ca2+ for cardiac contraction. R2920Q, a heretofore uncharacterized RyR2 mutation, was identified in a patient with family history of sudden death, syncope and prolonged QT interval. Methods & Results: We generated a mouse line harboring the RyR2-R2919Q mutation (R2920Q in human) using CRISPER technology. Mice heterozygous and homozygous for the mutation had hemodynamic parameters indistinguishable to control littermates (WT) by echocardiography. On the other hand, QTc interval in surface ECG of anesthetized mice was prolonged in homozygous mice compared to WT (76±0.6 and 57±0.6 ms, respectively). Correspondingly, at the cellular level, ventricular myocytes of homozygous mice had prolonged action potential duration (APD75 = 118±27 and 44±19 ms, respectively). At the molecular level, we are using [3H]ryanodine binding assays and Western blots to determine intrinsic properties of the channel protein. Conclusion: We identified a novel RyR2 mutation from a patient with LQT syndrome that resulted in LQT phenotype in mice. We are analyzing the mechanisms by which this mutation leads to LQT instead of the "classical" RyR2-linked arrhythmogenic syndrome CPVT.

ST09

Unraveling the role of Desmin in cardiac transdifferentiation, maturation and chromatin architecture

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The limited regenerative capacity of the heart and the insufficient therapeutic approaches, have increased the interest and efforts towards more efficient cardiac regeneration. Important steps have been achieved with the direct reprogramming, where fibroblasts transdifferentiate into functional induced cardiomyocytes (iCMs) through ectopic expression of three transcription factors GATA4, Mef2C and Tbx5, however with rather low efficiency. In our efforts to increase the transdifferentiation efficiency we turn to desmin, the major intermediate filament protein of cardiomyocytes. Our previous studies have proposed that the desmin intermediate filament network links the contractile apparatus to both the extracellular and nuclear matrix facilitating trafficking processes and mechanotransduction during development and adulthood. Indeed, we found that ectopic expression of desmin together with the GMT factors increases reprogramming by 40-60%. To elucidate the mechanism underlying this improvement, we investigate the genetic profile of iCMs in the presence or absence of desmin. Gene expression analysis has suggested that genes involved in major developmental pathways are timely regulated by desmin. In agreement, Chromosome Conformation Capture (Hi-C) analysis demonstrates the importance of desmin cytoskeleton on the genome 3D organization. We show that desmin is expressed in most of the cardiac progenitor cells population declaring its role in proper cardiac formation. Most importantly, our studies establish desmin as a major player for cardiac maturity, a critical subject in cardiac regenerative medicine, since its absence substantially reduces expression and proper localization of cardiac-specific proteins, delays myofibril formation and impairs the function of the induced cardiomyocytes driving most of them to senescence.

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ST10

Proinflammatory T Cells with Downregulated Unfolded Protein Response Genes Contribute to Experimental Heart Failure with Preserved Ejection Fraction (HFpEF)

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Heart Failure with Preserved Ejection Fraction (HFpEF) is an uncurable widespread syndrome characterized by diastolic dysfunction, preserved cardiac contractility, and downregulation of cardiomyocyte unfolded protein response (UPR). While T cell inflammation is known to contribute to other HF etiologies, whether T cell inflammation occurs in HFpEF remains elusive. Downregulation of the T cell UPR has been reported to improve T cell anti-tumor immunity. We hypothesized T cells contribute to diastolic dysfunction in HFpEF following T cell UPR downregulation in response to metabolic and nitrosative stress. We modeled cardiometabolic HFpEF in male C57/Bl6 (WT) and T cell receptor-alpha (Tcra-/-) mice using high-fat diet (HFD) and L-NAME-supplemented drinking water to mimic obesity and hypertension, respectively. Mice fed standard chow (STD) were used as controls. Using flow cytometry, we observed significant increases in cardiac CD4+ T cells in WT mice fed HFD/L-NAME, concordant with diastolic dysfunction and preserved EF, compared to STD mice. Tcra-/- mice fed HFD/L-NAME did not develop diastolic dysfunction or cardiomyocyte hypertrophy. Strikingly, qPCR analysis of splenic T cells of WT HFD/L-NAME mice revealed significantly decreased expression of spliced X box-binding protein 1 (XBP1s), activating transcription factor 4 (ATF4), and ATF6, effectors from all three UPR pathways, as well as their downstream targets, compared to controls. Our data demonstrate that diastolic dysfunction and cardiomyocyte hypertrophy are T cell dependent in preclinical cardiometabolic HFpEF and reveal downregulation of the T cell UPR as a potential novel mechanism that contributes to T cell inflammation in HFpEF.

ST11

Cell-type-specific gene expression and transcriptional networks reveal Adamts2 as a robust regulator of cardiac homeostasis during heart failure.

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Heart Failure (HF) is a highly heterogenous disorder characterized by interactions of multiple genes and cell types. By utilizing cell-type-specific gene expression paired with transcriptomic data from a large cohort of mice, we sought to identify key regulators of HF progression using a systems genetics approach.

Mice from 93 inbred lines of the Hybrid Mouse Diversity Panel were given 30 ug/g/day of isoproterenol for three weeks via osmotic minipump to induce HF. Transcriptomes were obtained from these mice and the weighted Maximal Information Component Analysis (wMICA) algorithm was used to generate gene co-expression networks.

Cardiomyocytes, Fibroblasts, and Endothelial Cells were isolated from control and isoproterenol-treated adult C57BL/6J hearts via Langendorff (n=3 per sex/treatment) and transcriptomes generated. Significantly differentially expressed genes were identified using DESEQ2 and overlaid on the wMICA-derived networks. Driver gene analysis identified Adamts2 as a likely regulator of cardiac hypertrophy.
Follow-up in vitro and in vivo work has demonstrated that Adamts2 knockdown significantly blunts the hypertrophic effect of isoproterenol on cardiomyocytes while simultaneously reducing fibroblast proliferation and increasing apoptosis as measured by TUNEL staining. Examination of the gene network reveals paracrine signaling between cardiomyocytes and fibroblasts and cross-cell type regulation of gene expression by Adamts2 knockdown.

These results suggest that Adamts2 plays an important role in HF progression via paracrine signaling in the proliferative response of fibroblasts and the hypertrophic response of cardiomyocytes to catecholamines. Further mechanistic analysis of Adamts2 will further reveal its role in the progression of heart failure.

ST12

Reduced CaMKII signaling in sinoatrial node from heart failure mice

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Heart failure (HF) is a complex syndrome in which death rates are over 50%. The main cause of death among HF patients is pump failure and ventricular arrhythmias, but severe bradycardia is also a common cause of sudden cardiac death, pointing to sinoatrial node (SAN) dysfunction. SAN pacemaker activity is regulated by voltage-clock and Ca2+-clock mechanisms and, although voltage-clock dysfunction in SAN has been largely proved in HF, Ca2+-clock dysfunction mechanisms in SAN remains unraveled.

Here we used a HF model in mice with transverse aortic constriction (TAC) and we saw by telemetry slower heart rhythm under autonomic nervous system blockade. Then by confocal microscopy we analyzed Ca2+ handling in HF SAN tissue and we found that intracellular Ca2+ transients rate were slower together with less frequency of Ca2+ sparks than in SHAM SAN tissue. Next, we studied protein expression of key excitation-contraction coupling proteins and we found reduced expression of the Na+-Ca2+ exchanger and reduced phosphorylated status of ryanodine receptor and phospholamban in the CaMKII sites for the SAN in TAC mice. Finally, the application of the CaMKII inhibitor, KN93, caused less effect in slowering the Ca2+ transient rates in HF SAN tissue, confirming the reduced CaMKII activation.

In conclusion, our data demonstrates a reduction in CaMKII activation in the Ca2+-clock function of the SAN tissue in a mouse model of HF.

ST13

Spurious Intragenic Transcription in Heart Failure Pathogenesis

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Using deep sequencing combined with computational biology, we found that the failing human heart contains a significant amount of aberrant transcripts arising from widespread spurious intragenic transcription initiations. The same observation was detected in failing mouse hearts induced by pressure overload and ischemia injury, suggesting a conserved phenomenon between murine and human heart failure (HF). The SET domain containing 2 (Setd2), an H3K36 methyltransferase, has been identified as a key regulator for transcription fidelity. Setd2 prevents RNA polymerase II (Pol II) entry from the gene body and subsequent cryptic transcription initiation. We analyzed the expression level of SETD2 and H3K36me3 and found that they were significantly reduced in the same cohort of failing human and mouse hearts. To elucidate whether loss of Setd2-mediated transcription fidelity is a causative mechanism promoting HF, cardiac-specific Setd2-knockout mice were generated. The mice developed a typical HF progression phenotype: initial compensatory hypertrophy followed by a gradually deteriorating HF with chamber dilation. Transcriptome analysis revealed activation of spurious intragenic transcription in the Setd2-/- cardiomyocytes prior to hypertrophy. DECAP-seq identified a significant increase in new intragenic transcription initiation sites, confirming that the increased aberrant transcripts resulted from spurious Pol II entry. Ribosome footprinting assay detected a significant amount of aberrant transcripts were translated into micro peptides, indicating that the accumulated aberrant transcripts and micro peptides together promote cardiac hypertrophy and eventual HF. Consistently, SETD2 overexpression repressed TAC-induced spurious transcripts. The transgenic mice were resistant to pressure overload with less cardiac hypertrophy and improved cardiac contractility. We report a new mechanism of HF pathogenesis, in which the dysregulated transcription fidelity causes adverse aberrant transcripts leading to cardiac hypertrophy and heart failure.

ST14

Cardiomyocyte proliferation is suppressed by ARID1A-mediated YAP inhibition during cardiac maturation.

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Shortly after birth, mammalian cardiomyocytes lose their proliferative ability and switch to a mature, non-regenerative state. The exact epigenetic mechanisms regulating proliferation and maturation gene programs are not fully understood. Arid1a, , a subunit of the switching defective/sucrose non-fermenting (SWI/SNF) chromatin remodelling complex, is a regulator of proliferation and differentiation, however, its roles during cardiac maturation are unknown. Here, we aim to identify the role of Arid1a as a potential regulator of neonatal cardiomyocyte maturation.

Using mouse models with perinatal ablation of Arid1a we show that cardiomyocyte-specific loss of Arid1a leads to severely malformed hearts at postnatal day 14. Arid1a mutant hearts displayed increased cardiomyocyte proliferation and reduced maturation. Genome wide transcriptome and epigenome analyses revealed that Arid1a promotes DNA access to transcription factors that drive a cardiomyocyte maturation gene program. Furthermore, we show that ARID1A directly binds and inhibits the proliferation-promoting transcriptional coactivators YAP and TAZ, indicating ARID1A sequesters YAP/TAZ from their DNA-binding partner TEAD. During ischemic heart disease, Arid1a expression is specifically enhanced in cardiomyocytes of the border zone region. Inactivation of Arid1a after ischemic injury enhanced proliferation of border zone cardiomyocytes, uncovering Arid1a as a suppressor of adult cardiomyocyte regeneration.

Our study demonstrates that Arid1a is crucial for cardiomyocyte maturation activation through ARID1A-mediated YAP inhibition. In addition, ARID1A suppresses cardiomyocyte proliferation after ischemic injury, which can be alleviated with its inhibition. Understanding the ARID1A regulated molecular pathways during cardiomyocyte maturation could uncover novel therapeutic strategies to alleviate suppression of cardiomyocyte proliferation for cardiac regenerative applications.

ST15

Transcription factor HIF-1α limits cardiomyoblast proliferation in a pluripotent stem cell model of human cardiomyogenesis.

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Background: The mechanisms controlling cardiomyocyte proliferation in development and regeneration are incompletely understood. Recent studies link proliferation to transient induction of glycolysis, through hypoxia inducible factor 1α (HIF-1α). However, whether induction of glycolysis by HIF-1α is oxygen-dependent, and whether it promotes or represses proliferation, remain controversial.

Objectives: To determine the role of HIF-1α in human cardiomyogenesis.

Methods: Using CRISPR-Cas9, we knocked-out HIF-1α in human induced pluripotent stem cells (HIF1α-KO hiPSCs). Wild-type (WT) and HIF1α-KO hiPSCs were subjected to stage-specific cardiomyocyte differentiation. Metabolic profiles were studied through gas chromatography- mass spectrometry (GC-MS). Proliferation was evaluated by flow cytometry, confocal microscopy, and immunoblotting.

Results: Immunoblotting and confocal analyses demonstrated robust, oxygen-independent induction of HIF-1α, which peaked on differentiation day-7 in wild type (WT), but not HIF1α-KO, hiPSC-derivatives. GC-MS showed increased lactate ($p=0.004$) and decreased D-Glucose ($p=0.02$) extracellular levels in WT vs. HIF1α-KO hiPSC-derived cardiomyocytes. Moreover, several Krebs cycle intermediates, with known prolyl hydroxylase domain (PHD) inhibitor activities, were increased in WT vs HIF1α-KO cells ($p<0.05$). Propidium iodide flow cytometric analysis demonstrated ~43% increase in G2M content on day-7 HIF1α-KO vs WT hiPSC-cardiomyoblasts. The enhanced proliferation of HIF1α-KO cardiomyoblasts was confirmed through PCNA immunoblotting; as well as confocal quantification of serine-10 phosphorylated Histone H3 ($p<0.001$).

Conclusions: Collectively, our results indicate that, during normal human cardiomyogenesis, HIF-1α is transiently stabilized through oxygen-independent mechanisms involving inhibition of PHDs by Krebs cycle intermediates; and induces glycolysis to limit cardiomyoblast proliferation. These findings have important therapeutic implications for stimulating cardiomyocyte regeneration in response to heart disease.

P01

Aortic valve neocuspidization using the Ozaki technique: A meta-analysis of patient-level data

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Background: Aortic valve neocuspidization using the Ozaki technique has shown promising results both in adults and children. We sought to synthesize all available evidence on the hemodynamic benefit and durability of the Ozaki procedure.

Methods: A systematic search of the PubMed and Cochrane databases was performed up to November 13th, 2021. Individual patient data were reconstructed and analyzed from the Kaplan-Meier curves of all eligible studies for time-to-event outcomes.

Results: We included a total of 22 studies reporting on 1,891 patients who underwent Ozaki repairs. The most common indication was aortic stenosis (46.4%, 95% CI 34.1 - 58.6). Mean cross-clamp and cardiopulmonary bypass duration were 106.8 ± 24.8 min and 135.2 ± 35.1 min, respectively. Permanent pacemaker was required in 0.7% (95% CI 0.4 – 1.2) of the patients. At discharge, mean effective orifice area was 2.08 ± 0.5cm2/m2. At latest follow up, peak gradient was 15.7 ± 7.4 mmHg and only 0.25% (95% CI 0 – 2.3) had moderate aortic insufficiency. In-hospital mortality was 0.67% (95% CI 0.07–1.68). At a mean follow up of 38.1 ± 23.8 months, mortality and freedom from reintervention were 1.91% (95% CI 0.19–4.72) and 96.7.% (95% CI 94.0 - 98.7), respectively. More than half of the reoperations were due to infective endocarditis (51.5%, 95% CI 18.3 – 84).

Conclusions: The midterm outcomes of the Ozaki procedure are excellent in terms of hemodynamics, survival, and freedom from reoperation. Acquiring long-term follow-up will help solidify this technique in the cardiac surgery armamentarium.

P02

EPAS1 directs a network of genes implicated in mitochondrial dysfunction in arrhythmogenic cardiomyopathy

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Genetic variants in desmosomal genes are linked to arrhythmogenic cardiomyopathy (ACM), a disease characterized by arrhythmias and cardiomyocyte death. Despite the fact that the pathophysiology of ACM is well described, the molecular mechanisms inciting cardiac cell death are poorly understood.

In an effort to identify driving mechanisms of disease we performed spatial transcriptomics on the left ventricular free wall of a heart explanted from an ACM patient carrying a mutation in desmoplakin (DSP p.Lys569X/WT). Hierarchal clustering revealed stressed regions within the myocardium to be enriched for genes related to mitochondrial dysfunction, including BNIP3, BNIP3L and SOD2. Examination of the DNA-binding motifs surrounding these genes identified endothelial PAS domain-containing protein 1 (EPAS1) as potential regulator. To substantiate these results we explored the molecular and functional landscape in mutant human induced pluripotent stem cell derived cardiomyocytes (DSP p.Arg1113X/WT). EPAS1 expression was induced in mutant cardiomyocytes, which was accompanied by increased levels of BNIP3L and SOD2. Functionally, we observed impaired mitochondrial function in mutant cardiomyocytes, as demonstrated by a reduced oxygen consumption rate, along with elevated reactive oxygen species levels. Overexpression of EPAS1 in control cardiomyocytes resulted in upregulation of BNIP3L and SOD2, corroborating the observed molecular link between these proteins. Importantly, we determined the levels of EPAS1 and BNIP3L in additional human explanted ACM hearts, revealing an induction of these proteins when compared to control hearts.

Together, our findings uncover a role for EPAS1 in the regulation of mitochondrial function during ACM pathogenesis. Future studies will focus on the underlying molecular mechanisms.

P03

Restoring levels of PKP2 as a novel therapeutic approach for Arrhythmogenic cardiomyopathy

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Arrhythmogenic cardiomyopathy (ACM) is a severe cardiac disorder characterized by lethal ventricular arrhythmias and sudden cardiac death (SCD). To date, there is no effective curative treatment for the disease. ACM patients often carry mutations in desmosomal genes, with Plakophilin 2 (PKP2) being the most prevalent. Our lab has shown that most of the patients harboring a PKP2 mutation, exhibit reduced levels of PKP2 protein, which corresponds to a lowering in other desmosomal components and the dysregulation of ACM-related factors, such as beta-catenin and FHL. These data suggest that point mutations in PKP2 weaken desmosomal assembly, which subsequently disrupts the connection between cardiomyocytes and activates downstream disease processes. To further explore these ACM aspects, our lab has generated multiple in vitro and in vivo knock-in models harboring disease-associated point mutations.

Since PKP2 haploinsufficiency is the underlying cause of ACM, we hypothesized that restoration of PKP2 levels would reverse the phenotype caused by PKP2 mutations. To do so, we used AAV-mediated overexpression of wildtype PKP2 in our preclinical models of ACM. Restoring PKP2 back to physiological levels in hiPSC-CMs carrying the human pathogenic PKP2 c.2013delC mutation resulted in subsequent recovery of DSP, PKG and DSG protein levels. Importantly, this result was corroborated by intravenous administration of AAV9-PKP2 to mutant mice harboring the equivalent of the human pathogenic PKP2 mutation.

These findings suggest that restoration of PKP2 levels in ACM patients carrying a pathogenic PKP2 mutation could reverse the disease phenotype and as such be of therapeutic value in this patient population.

P04

Circadian regulation of mitophagy and mitochondrial function in the heart

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Prior studies have indicated that mitochondrial function is perturbed in the hearts of mice carrying aberrant circadian clock regulators. Furthermore, some of the key proteins involved in mitochondrial fission and fusion, and mitochondrial quality control (autophagy of the mitochondria or mitophagy) are also circadian-dependent. We have performed studies to determine whether mitophagy is regulated by fasting and/or the circadian clock in the heart with the following findings. Using transmission electron microscopy, we found that mitochondrial number is regulated by both time-of-the-day and fasting, and that this time-of-day regulation is sex-dependent. Using confocal microscopy, we measured mitophagy at different times of the day using Mito-QC mice (which contain a protein targeted to the mitochondria which exhibit both red and green fluorescence in the mitochondria and when mitophagy occurs and part of the mitochondrion is translocated to the lysosomes only red fluorescence remains). Our results demonstrated time-of-day regulation of mitophagy which was also sex-dependent. Furthermore, using Seahorse XF analyses, we found that mitochondrial electron transport chain activities are sex-dependent and significantly decreased by fasting. These studies provide new insights on circadian regulation of mitochondrial function and quality control in the heart.

P05

M6A RNA methylation as a mediator of heart response to mechanical overload

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INTRODUCTION
Heart failure (HF) remains a leading cause of morbidity and mortality worldwide. The heart is exposed to mechanical forces and constantly adapts to its environment in a process known as cardiac remodelling. Excessive mechanical load is a major driver of pathological cardiac remodelling, leading to HF. A set of microRNAs (miRs) are responsive to mechanical forces (mechano-miRs) and contribute to heart pathophysiology and HF.
Epitranscriptomics (i.e., the biochemical modification of the RNA) is emerging as a new layer of gene expression regulation affecting both coding and non-coding RNAs. N6-adenosine RNA methylation (m6A) regulates the processing of miR transcripts (pri-miRs) to functional miRs, promoting angiogenesis and cardiac repair after myocardial infarction. However, dysregulation of m6A reportedly contributes to HF. The role played by epitranscriptomics in heart mechanical responses and mechano-miR regulation has not yet been investigated.
This project aims to unveil the mechanically induced epitranscriptomic changes leading to HF, focussing on mechano-miRs.

METHODS and RESULTS
Adopting living myocardial slice (LMS) technology to model the effects of mechanical stress (volume overload) on the heart in HF, we observed dysregulation of global m6A levels both in rat LMSs exposed to mechanical overload and in human HF. Using bioinformatic methods, we have analysed and integrated published RNA-seq data (GEO) on mechanically stimulated cardiomyocytes and our small RNA-seq data on HF patients undergoing aortic valve replacement surgery (vs respective controls). This led us to identify a novel group of potentially mechanosensitive miRs (mechanomiRs), whose expression is consistently downregulated upon mechanical overload in different models and species (human, rat, mouse). Using meRIP-PCR, we are investigating the N6-methylation profile of the pri-mechanomiRs and mechanomiR-mRNA targets.

CONCLUSION
Mechanical overload affects m6A level and the cardiac expression of mechanosensitive miRNAs. The discovery of new mechanically regulated m6A events could contribute to designing potentially transformative therapeutic strategies.

P06

FIMICS: a panel of long noncoding RNA biomarkers for cardiovascular conditions

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Background. Long noncoding RNAs (lncRNAs) usually lack strong sequence conservation across species but are highly tissue-specific in nature. In recent years, lncRNAs have emerged as important biomarkers in various human diseases. Cardiovascular disorders such as coronary artery disease, acute myocardial infarction and heart failure (HF) are leading causes of mortality and morbidity in the modern world. Patient stratification via identification of novel biomarkers could improve management of cardiovascular diseases of complex etiologies. Single biomarker might not be useful for such complex pathology, so we focused on identification of a molecular signature for the cardiac pathophysiology.

Aim. In this study, we aimed to identify cardiac-enriched lncRNAs as potential biomarkers for HF and other cardiovascular conditions.

Methods and results. Deep RNA sequencing was performed on left ventricular biopsies obtained from 21 explanted failing hearts and 5 non-failing control hearts to analyse differentially expressed lncRNAs in heart failure. An independent RNA-seq dataset was used to evaluate the enrichment of lncRNA expression in normal hearts. We identified 3233 lncRNAs that are either cardiac-enriched or associated with HF. Expression of selected lncRNAs was validated by quantitative RT-PCR in an independent cohort of 93 left ventricular biopsies from failing and non-failing human hearts. Expression of selected lncRNAs was also confirmed in blood and plasma samples from healthy donors. Further validation experiments conducted in whole blood samples showed that the panel could differentiate patients with acute myocardial infarction from control samples.

Conclusion. We have developed the FIMICS panel of 3233 lncRNAs, that are either cardiac-enriched or differentially expressed between failing and non-failing hearts. This panel could provide insights of cardiovascular health or pathology of an individual and could be helpful for diagnosis, monitoring and prognosis. The panel has strong potential to identify non-invasive biomarkers for personalized healthcare.

P07

A procedure for the in vitro evaluation of the immunosuppressive effect of mesenchymal stem cells

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Mesenchymal stem/stromal cells (MSC) represent adult cells with multipotent capacity. Besides their capacity to differentiate into multiple lineages in vitro and in vivo, increasing evidence points towards the immunomodulatory capacity of these cells, as an important feature for their therapeutic power. Although not included in the minimal criteria established by the International Society for Cellular Therapy as a defining MSC attribute, demonstration of the immunomodulatory capacity of MSC can be useful for the characterization of these cells before being considered MSC.

Here we present a simple and reliable protocol by which the immunosuppressive effect of mouse bone marrow-derived MSC can be evaluated in vitro. It is based on the measuring of the proliferation of activated T cells cultured in direct contact with irradiated MSC.

Our results showed that mouse MSC have a dose-dependent inhibitory effect on activated T cell proliferation, which can be quantified as a percentage of maximum proliferation. The immunosuppressive properties of MSC vary between batches, but not between different passages of the same batch. The batch-to-batch variability can be determined within one or multiple experiments, by extracting the area under curve of T cell proliferation plotted against the absolute number of MSC in co-culture.

In conclusion, the validation of the immunosuppressive capacity of MSC could be added to the characterization of the cells before being used in various MSC-based approaches to treat immune mediated diseases.

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P08

Single-cell transcriptomics provides insights into hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a heart disease characterized by unexplained hypertrophy. While sarcomeric gene mutations are often the basis for HCM, the mechanistic origin for the remodeling remains largely unknown. A better understanding of gene networks driving HCM is required to improve therapeutic strategies. Here, we used single cell RNA sequencing (scRNA-seq) to study cellular transcriptional differences between healthy and hypertrophic cardiomyocytes (CMs) and leveraged expressional heterogeneity to link gene expression profiles to cellular characteristics related to HCM. Specifically, we performed scRNA-seq on tissue samples from HCM patients that received septal myectomy surgery, which is a procedure to relieve outflow tract obstruction due to hypertrophy. In addition, we included healthy cardiomyocytes from previous scRNA-seq studies as a reference. In depth analysis of the scRNA-seq data from HCM CMs indicated the presence of subpopulations of CMs to which each patient contributed. Additionally, we were able to identify HCM-related gene correlations. We identified multiple HCM-enriched groups of genes that are co-expressed (regulons) and we were able to link these to transcription factors (TFs) that are potentially responsible for their activation. Additionally, index-sorting data enabled us to correlate gene expression profiles to CM hypertrophy, of which we confirmed the gene MYL2 to be enriched in larger CMs. Together these data indicate CM heterogeneity in the human heart and show that scRNA-seq provides insights into cellular and molecular mechanisms that are potentially relevant for HCM.

P10

Local microenvironment activates in vivo apoptosis of mesenchymal stromal cells after transplantation

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Background. Mesenchymal stromal cells (MSC) are attractive tools for cell-based therapy, yet the mechanisms underlying their migration and survival post-transplantation are unclear. Accumulating evidence indicates that MSC apoptosis modulates both innate and adaptive immune responses which impact on MSC therapeutic effects.

Methods. Using a dual tracking system, namely the Luciferase expression and VivoTrack680 labelling, and in vivo optical imaging, we investigated the survival and migration of MSC transplanted by various routes (intravenous, subcutaneous, intrapancreatic and intrasplenic) in order to identify the best delivery approach that provides an accumulation of therapeutic cells to the injured pancreas in the non-obese diabetic (NOD) mouse.

Results. The results showed that transplanted MSC had limited migration capacity, irrespective of the administration route, and were short-lived with almost total disappearance at 7 days after transplantation. Within one day after transplantation, cells activated hypoxia-signaling pathways, followed by Caspase 3-mediated apoptosis. These were subsequently followed by local recruitment of immune cells at the transplantation site, and the engulfment of apoptotic MSC by macrophages.

Conclusions. This study provides important insights regarding the fate of exogenously administered MSC in mice with autoimmune insulitis. Our results argue for a “hit and die” mechanism of transplanted MSC. Further investigations will elucidate the molecular crosstalk between the inoculated and the host-immune cells.

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P11

Mechanical load affects the regulation of contractility of myocardial slices via extracellular vesicles

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Mechanical load is determinant in maintaining cardiac output (CO). Extracellular vesicles (EVs) released in response to changes in CO are important mediators of the cardiac intercellular communication. Myocardial slices are organotypic preparations that offer the unique opportunity to study the paracrine crosstalk mediated by EVs in the cardiac hetero-cellular environment.
Purpose: to assess whether mechanical load affects the regulation of contractility via extracellular vesicles.
Slices were prepared from rat left ventricle using a vibratome and stretched uniaxially at a sarcomere length (SL) of 2.2µm to recapitulate physiological load. After 48-hours culture under electromechanical stimulation in M-199 media with Earls' salts, catecholamines, dexamethasone and triiodothyronine, force-stretch relationship was performed, and slices' media harvested and processed for exosomes isolation by size exclusion chromatography. Physiological EVs were then applied onto overloaded slices stretched at 2.4 µm SL (1x108 particles/slice), and force-stretch relationship assessed after 48-hours.
Overloaded slices treated with physiological slices derived-EVs showed a significant increase in contractility from 20-30% stretch compared to untreated slices ($p<0.05$, $p<0.001$, $p<0.0001$ at 20%, 25% and 30% stretch, respectively); (n=15 EVs-treated slices, n=19 control slices). Moreover, EVs-treated slices showed a significant increase in the time to peak ($p<0.05$), time to 50% ($p<0.01$) and time to 90% decay ($p<0.05$) at 30% stretch, compared to controls.
EVs derived from physiologically loaded slices improve the contractility of overloaded slices after 48-hours. Understanding the mechanisms by which the EVs released in response to changes in load affect cardiac contractility may reveal interesting targets for the treatment of heart failure.

P12

Drug Screening in Human iPSC-Cardiomyocytes Identifies Serine Biosynthesis Pathway as a novel Therapeutic Target for Dilated Cardiomyopathy

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Background: Genetic dilated cardiomyopathy (DCM) is a leading cause of heart failure. Despite significant progress in understanding the DCM genetic etiologies, the molecular mechanisms underlying the pathogenesis remain unknown, translating to a lack of disease-specific therapies.

Methods and Results: Using patient-specific iPSCs carrying a pathogenic TNNT2 gene mutation and CRISPR-based genome editing, we developed a faithful DCM model in vitro. We performed a phenotypic screening in TNNT2 mutant iPSC-derived cardiomyocytes (iPSC-CMs) with small molecule kinase inhibitors (SMKIs). We discovered a dual SMKI treatment, combining Gö6976 and SB203580, that rescued contractile dysfunction in DCM iPSC-CMs carrying gene mutations of various ontologies (TNNT2, TTN, LMNA, PLN, TPM1, LAMA2). Transcriptomic analysis revealed that combinatorial SMKI treatment upregulated the expression of genes related to serine, glycine, and one-carbon metabolism, and significantly increased the intracellular levels of glucose-derived serine and glycine in DCM iPSC-CMs. Furthermore, dual SMKI treatment rescued mitochondrial respiration, tricarboxylic acid cycle metabolites, and ATP production. Finally, we show that the phenotypic rescue was mediated by the activating transcription factor 4 (ATF4) and its downstream genes, phosphoglycerate dehydrogenase (PHGDH), which encodes a critical enzyme of the serine biosynthesis pathway, and Tribbles 3 (TRIB3), a pseudokinase with pleiotropic cellular functions.

Conclusions: We established a phenotypic screening platform in iPSC-CMs for therapeutic target discovery. The dual SMKI treatment ameliorated contractile and metabolic dysfunction in DCM iPSC-CMs via serine biosynthesis pathway and TRIB3 signaling. Together, our findings suggest that manipulation of serine biosynthesis and TRIB3 signaling may represent a novel, genotype-agnostic therapeutic strategy for genetic DCM.

P13

MiRNAs generated from Meg3-Mirg locus are downregulated during aging

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Aging determines a multilevel functional decline and increases the risk for multiple pathologies, including cardiovascular disorders. MicroRNAs are recognized as fine tuners of all cellular functions, being important players in the maintenance of homeostasis and adaptability to stress factors accumulated during the aging process.

The aim of this work was to advance the understanding of aging process by identification of the aging-associated miRNA profile in cardiac fibroblasts.

To this aim, cardiac fibroblasts were isolated from young (2-3-mo old), old (16-18-mo old), and very old (23-24-mo old) mice, through enzymatic digestion of the cardiac ventricles followed by 5-day culture in the presence of FGFβ and insulin. miRNA expression profile was assessed in young and old mesenchymal cells by next-generation sequencing of small RNA and the results were further confirmed in cells and organs obtained from very old group by qRT-PCR analysis.

Bioinformatic analysis of the next-generation sequencing revealed that most downregulated miRNAs were mapped to chromosome 12, as part of Meg3-Mirg locus. To address the specificity of this repression, four miRNAs were selected as representative for this locus and further assessed in cardiac cells and organs harvested from young, old and very old groups. The results suggested that the repression of miRNAs generated by the Meg3-Mirg locus was as a general feature of aging. Besides, all long non-coding RNAs within the locus were also found downregulated in aged ventricles.

We therefore advance the hypothesis that the downregulation of Meg3-Mirg locus is an age-dependent response with biological significance in aging and aging-associated disorders.

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P14

Deletion of MyD88 in T-cells Worsens Non-Ischemic Heart Failure Through Enhanced T-cell Survival and Induction of Cardiac Fibrosis

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Heart failure (HF) is a leading cause of death worldwide, associated with cardiac and systemic inflammation. However, anti-inflammatory therapies have shown limited success. Damage associated molecular patterns (DAMPs) released in the heart activate myeloid cells through the adaptor "Myeloid differentiation response 88" (MyD88) promoting antigen presentation to T-cells. T-helper cells (Th1) infiltrate the heart and induce cardiac fibrosis through mechanisms involving adhesion to cardiac fibroblasts (CFB) via vascular adhesion molecule-1 (VCAM-1). MyD88 is also expressed in cells of the adaptive immune system, however its' function is not well understood. To investigate the role of T-cell MyD88 in experimental HF, we reconstituted Tcra-/- mice, normally protected from HF induced by transaortic constriction (TAC), with WT or Myd88-/- Th1 cells in the onset of TAC. We found that mice receiving Myd88-/- Th1 cells exhibited increased cardiac fibrosis and T-cell presence compared to mice receiving WT cells. Mice with a T-cell specific deletion of Myd88 (Myd88fl/fl-CD4Cre) exhibited similarly increased cardiac fibrosis and T-cells. We found that Myd88-/- Th1 cells showed increased survival both in vitro as measured by propidium iodide incorporation and in vivo using competitive survival analysis in Tcra-/- mice. We then found increased adhesion ability to immobilized VCAM-1 in Myd88-/- Th1 cells compared to WT Th1 cells, which resulted in increased binding to and transformation of CFB in culture. Together these data demonstrate that MyD88 limits T-cell mediated cardiac fibrosis in HF by regulating T-cell survival and fibroblast transformation, providing a novel role for T-cell MyD88 in cardiac inflammation.

P15

VAPB Physically Interacts with Phospholamban in Mouse Ventricular Cardiomyocytes

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The sarcoendoplasmic reticulum (SER) is the primary organelle facilitating intracellular Ca2+ cycling in cardiomyocytes, ensuring proper excitation-contraction coupling (ECC). Tight coupling between membrane potential and SER-mediated Ca2+ release occurs at discontinuous membrane contacts -junctional membrane complexes (JMCs) - between the SER and T-tubules, stabilized by tethering proteins. Disruption of these JMCs results in T-tubule disorganization, ECC loss of function, and heart failure. Junctophilin-2 is a key structural protein tail-anchored in the SER membrane tethering it to T-tubule membrane invaginations. However, other tethering proteins and their potential role in cardiac ECC have not been studied. Using mass-spectrometry-based complexome profiling and co-immunoprecipitation assays, we found an interaction between a major phospho-regulatory SER protein, phospholamban (PLN), and the tethering protein Vesicle-Associated membrane protein-associated Protein B (VAPB) in murine ventricular cardiomyocytes. VAPB has been implicated in regulating a variety of cellular processes including, microtubule organization, Ca2+ homeostasis, and ER stress and the unfolded protein response (UPR). Motoneuronal cells with a P56S or a T46I mutation in VAPB failed to activate the UPR pathway, resulting in the formation of ubiquitinated aggregates. Additionally, a previous study has shown that VAPB-/- knockout mice developed cardiac bradycardia and delayed ventricular repolarization suggesting a physiological role for VAPB in intracellular cardiomyocyte Ca2+ homeostasis (PMID: 29879376). Among the established protein-protein interactions, those formed between VAPB, beta-tubulin and the UPR are of high biomedical interest in cardiomyocytes. In particular, we hypothesize that the loss of interaction between PLN and VAPB results in disrupted intracellular Ca2+ signaling and UPR activation.

P16

Mitochondrial dysfunction as an underlying cause for arrhythmogenic cardiomyopathy

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Arrhythmogenic cardiomyopathy (ACM) is a rare cardiac disease characterized by arrhythmias and progressive fibrofatty replacement of the myocardium, ultimately leading to heart failure. It is one of the leading causes of sudden cardiac death in young adults, especially athletes. The genetic cause is well studied where the majority of ACM patients carry a mutation in desmosomal genes. However, the molecular disease-driving mechanisms remain poorly understood.

To study ACM, human induced pluripotent stem cells (hiPSCs) generated from an ACM patient carrying a pathogenic mutation in the desmosomal gene Plakophilin-2 (PKP2-c.2013delC/WT) were differentiated into cardiomyocytes (hiPSC-CMs). These mutant hiPSC-CMs show haploinsufficient PKP2 levels, decreased desmosomal protein expression and a reduced sodium current compared to their isogenic control; recapitulating the molecular and electrophysiological phenotype in ACM patients. Furthermore, RNA-sequencing showed that oxidative-phosphorylation-related genes were downregulated in the mutant cells. This decline corresponded to reduced ATP production and respiratory capacity in the mutant cardiomyocytes. Using siRNA-mediated PKP2 inhibition in control hiPSC-CMs, we demonstrated that this decreased expression of ETC components and loss in mitochondrial function was a direct consequence of PKP2 depletion and occurred in a dose-dependent manner. We are currently investigating the effect of oxidative stress in an ACM mouse model (PKP2-c.2013delC equivalent).

Our findings demonstrate that a decline in PKP2 levels alters gene expression of ETC components that consequently cause mitochondrial dysfunction, indicating a possible increased susceptibility for oxidative stress in ACM patients. Therefore, improving mitochondrial function in cardiomyocytes could be a new potential therapeutic strategy for patients suffering from the disease.

P17

PPARβ/δ attenuates cardiac tissue damage by regulating mitochondrial quality control and inhibiting inflammation in a genetic animal model of heart failure

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PPARβ/δ is a major transcription regulator of cardiac energy metabolism with anti-inflammatory, anti-oxidative properties and cardioprotective action. In this study, we sought to investigate whether pharmacological activation of PPARβ/δ could ameliorate cardiac tissue damage in desmin null mice (Des-/-), a genetic model of heart failure and explore the potential effects on the impaired mitochondrial homeostasis. Damaged mitochondria, extensive cardiomyocyte death, an early acute inflammatory response and severe cardiac remodeling lead to dilated cardiomyopathy and eventually heart failure in Des-/- mice. Our findings demonstrate that PPARβ/δ activation alleviates cardiac inflammation, fibrosis and cardiac remodeling, all hallmarks of the Des-/- heart. Moreover, PPARβ/δ activation ameliorates oxidative stress in the failing myocardium as evidenced by decreased superoxide levels. Importantly, PPARβ/δ activation stimulates mitochondrial biogenesis, prevents mitochondrial vacuolar degeneration and improves the deranged mitochondrial network as observed in transmission electron microscopy images. Concomitantly, PPARβ/δ promotes the balance between fission/fusion markers and enhances mitochondrial functionality in Des-/- hearts. In conclusion, PPARβ/δ activation exerts protective effects during myocardial degeneration and heart failure in Des-/- hearts by preserving the structural and functional quality of the mitochondrial network and attenuating inflammation. These findings implicate PPARβ/δ for further clinical development.

P18

Evaluation of DOX-Induced Cardiotoxicity by miR-484 Expression in Breast Cancer Patients

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Doxorubicin (DOX), an anthracycline anticancer drug, is an effective chemotherapeutic against various cancer types. It may lead to the production of reactive oxygen species, increase mitochondrial dysfunction, but it may cause cardiomyopathy[1]. Cardiomyopathy is one of the fatal adverse effects of DOX[2]. miRNAs play critical roles in cell development, differentiation, and apoptosis. Deregulation of miRNAs emerges as biomarkers in many diseases. For example, high expression of miR-484 was reported in cardiomyocytes[3]. Therefore, the present study aims to study the roles of miR-484 in cardiotoxicity in breast cancer patients by comparing its expression levels between DOX-treated and untreated patients.

Total RNA was extracted from the blood samples of three different groups; DOX-treated, untreated (Paclitaxel or Herceptin-treated) patients, and healthy people (control group) (n=5). miR-484 expression was analyzed with RT-qPCR. One-Way ANOVA and Student's t-test were performed for statistical differences.

As a result, the relative expression of miR-484 in the DOX-treated group was 100% higher than in untreated samples. Moreover, the relative expression of miR-484 in the control group was two times higher than in untreated samples ($p<0.05$). Considering that each patient was DOX-treated for at least four chemotherapy sessions, this fold change may be a notable indicator for detecting DOX-induced cardiotoxicity in breast cancer patients like lung and thyroid cancer[4].

In a conclusion, miR-484 expression of DOX-induced cardiotoxicity might be a distinguished indicator for cardiomyopathy diagnosis during breast cancer treatment. This study may form the basis for future studies in the field of targeted therapies in the occurrence of chemotherapeutics-induced cardiomyopathy.

Keywords: DOX, Cardiomyopathy, miR-484, Cardiotoxicity

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P19

Dissecting phospholamban (PLN) p.Arg14del cardiomyopathies on the proteome level

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Objective
Phospholamban (PLN) p.Arg14del cardiomyopathies are characterized by misregulation of the endosarcoplasmic reticulum Ca2+ pump. Regulatory function of PLN on SERCA2a-mediated Ca2+ transport is largely controlled by phosphorylation of S16 and T17 residues. The p.Arg14del mutant abolishes S16 phosphorylation by removing the corresponding PKA-C recognition motif and reducing SERCA2a transport activity. We investigated the effect of the p.Arg14del mutation on functional interactions of PLN, on the global proteome and the phosphoproteome.
Methods
Cardiomyocyte and cardiac total membrane preparations from wildtype and heterozygous p.Arg14del mice and cardiac membrane prepations from human samples were isolated and analyzed by data-dependent analysis (DDA) and data-independent analysis (DIA) mass spectrometry on high resolution mass spectrometry platforms
Results
(i) AP-MS and APEX2-MS analysis in NRCMs pointed to 14-3-3 phosphoadaptor proteins binding to PLN in its pentameric state, blocking PP1c phosphatase access to S16/T17 and keeping SERCA2a in the active state. Lack of S16 phosphorylation reduces 14-3-3/pPLN binding.
(ii) BN-PAGE Complexome Profiling in mice identified a novel Ca2+ cycling PLN-RyR2-SERCA2a supercomplex withPPP1R3A. The PLN-RyR2-SERCA2a supercomplex was confirmed in healthy human control samples, however was disrupted in paroxysmal and chronic atrial fibrillation patients
(iii) DIA mass spectrometric profiling of p.Arg14del and wildtype mice shows significant downstream effects on the cardiac proteome, specifically on proteins of the OxPhos system and the inner mitochondrial membrane.
Conclusion
Mass spectrometric proteome analysis reveals a significant impact of the PLN p.Arg14del mutation on functional protein/protein interactions relevant to Ca2+ homeostasis and on global cardiac energy metabolism.

P20

The RNA-binding protein QKI governs a muscle-specific alternative splicing program that shapes the contractile function of cardiomyocytes

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The protein Quaking (QKI) is a splicing factor highly expressed in the heart that is dysregulated in heart failure. As an RNA-binding protein, QKI regulates cellular function by binding to target pre-mRNAs at specific sequence motifs and guiding its alternative splicing. The exact function of QKI in the heart is unclear since the specific splicing events controlled by QKI remain uncharacterized in cardiomyocytes.

In this project, we created a QKI cardiomyocyte-specific tamoxifen-inducible knock out model based on the Myh6-MerCreMer line. We found that QKI depletion in the adult heart induces a rapid and dramatic systolic dysfunction: up to a 50% reduction in ejection fraction one week after the tamoxifen injections. To characterize the specific splicing events that are regulated by QKI in cardiomyocytes, we performed RNA-Sequencing in left ventricle samples of these mice. QKI depleted hearts showed a loss of muscle-specific isoforms of hundreds of cardiac essential genes; including sarcomeric genes (e.g. Ttn, Nebl, Tpm1), cytoskeletal genes (e.g. Ank3, Dst, Ablim1) and calcium handling genes (e.g. Camk2d, Ryr, Atp2a2). Remarkably, important transcription factors (e.g. Mef2c), epigenetic regulators (e.g. Hdac4/5/9) and splicing regulators (e.g. Celf2, Mbnl2, Rbfox1/2) for cardiomyocyte function were also misspliced in the absence of QKI.

Altogether, our results indicate that QKI is a core regulator of the muscle-specific alternative splicing program that shapes the cardiomyocyte transcriptome. Further experiments will be performed to study how differences in QKI activity contribute to the molecular changes that occur in heart failure.

P21

Loss of a ttn-derived circRNA leads to sex-specific cardiac remodeling in mice

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Mutations in TTN are the most frequent cause of hereditary heart failure. Furthermore, TTN produces the highest number of circRNAs in the heart. We have shown that the subclass of RBM20-dependent TTN-derived circRNAs generates a specific functional sequence in the backsplice junction. Furthermore, we showed that a human specific circRNA of this subclass is essential for normal splicing of key muscle genes in hiPSC-CM. In this study, we further investigate the role of these circRNAs derived from the ttn in vivo.

We selected the highest expressed circRNA derived from ttn that is conserved between human and mouse (cttn4). We specifically downregulated this circRNA in mice by AAV9 delivered shRNAs directed against the backsplice junction. Loss of cttn4 resulted in mild cardiac hypertrophy with preserved ejection fraction in male mice, while in female mice ejection fraction was reduced without any signs of cardiac hypertrophy. Molecular analysis revealed a decrease in total ttn levels in male mice only, while ttn splicing was affected in both sexes leading to a downregulation of the ttn N2BA isoform. Interestingly, loss of cttn4 resulted in downregulation of the ttn splicing regulator RBM20 only in males. While no effect on RBM20 mRNA levels was detected in females, we detected an upregulation of two camk2d isoforms which are also RBM20 splice targets.

ShRNA-based selective loss of cttn4 in vivo results in a dimorphic response in male and female mice. These results suggests a sex-specific role of cttn4 in cardiac homeostasis. We are currently further investigating the role of cttn4.

P22

Aberrant PLN-R14del associations underlying impaired Ca2+ handling and arrhythmogenesis

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Arrhythmogenic cardiomyopathy is characterized by life-threatening ventricular arrhythmias and sudden cardiac death. Multiple genes have been implicated as the underlying genetic etiology of disease, including phospholamban (PLN). PLN is a key modulator of Ca2+ homeostasis that inhibits sarcoplasmic reticulum (SR) calcium ATPase (SERCA2a) and regulates cardiac contractility. A human PLN mutation causing heterozygous deletion of arginine at amino acid residue 14 (R14del) has attracted considerable attention, with R14del patients identified across the world. A humanized PLN-R14del mouse model has been shown to recapitulate human clinical features including Ca2+ defects, contractile dysfunction and increased susceptibility to arrhythmias.

In the present study, we examined for potential alterations in PLN-R14del associations to known PLN-interacting partners. By pull-down assays using PLN-WT or PLN-R14del recombinant proteins, PLN-R14del interactions to SERCA2 and HAX-1 were found to be enhanced. These alterations were confirmed by immunoprecipitations in PLN-R14del mouse hearts. As the inhibitory effect of PLN on SERCA2 activity is relieved upon phosphorylation, we subsequently examined the impact of phosphorylation on PLN-R14del/SERCA2 interaction. In contrast to PLN-WT, phosphorylation did not affect PLN-R14del binding to SERCA2a due to lack of Ser-16 phosphorylation in the PLN-R14del mutant. Concomitantly, at the physiological level, isoproterenol treatment did not stimulate contractility in cardiomyocytes from PLN-R14del mice, indicating failure of SERCA2 inhibition relief by the mutant protein.

In conclusion, our findings reveal that PLN-R14del acts as super-inhibitor of SERCA2a activity by increased binding to SERCA2a and HAX-1. These aberrations may underlie the pathological mechanisms contributing to impaired Ca2+-homeostasis and arrhythmogenesis in PLN-R14del hearts.

P23

A CRISPR/Cas9-induced mutant zebrafish line reveals the essential role of cfdp1 in cardiac development and function

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Cardiovascular diseases (CVDs) are the prevalent cause of mortality worldwide and account for the most common noncommunicable disease. CVDs describe a wide spectrum of disorders affecting the proper function, physiology and morphogenesis of the heart and blood vessels. The risk of developing cardiovascular diseases is modulated by a combination of environmental and genetic effectors. Thus, it's highly important to identify candidate genes and elucidate their role in the manifestation of the disease. Large-scale human studies have revealed the implication of Craniofacial Development Protein 1 (CFDP1) in coronary artery disease (CAD). CFDP1 belongs to the evolutionary conserved Bucentaur (BCNT) family and up to date, its function and mechanism of action in Cardiovascular Development is still unclear. In this study, we utilize zebrafish to investigate the role of cfdp1 in the developing heart due to the high genomic homology, similarity in heart physiology and the ease of experimentally manipulation. We showed that cfdp1 is expressed during development and at 120 hours post fertilization its expression is restricted to the region of the heart and the head. We then generated a cfdp1-null zebrafish line using CRISPR-Cas9 system which led to a lethal phenotype since knockout embryos do not reach adulthood. cfdp1-/- embryos develop arrhythmic hearts and defective cardiac performance exhibiting statistically significant differences in heart features including End Diastolic Volume, Cardiac Output, Ejection Fraction and Stroke Volume. Myocardial trabeculation is also impaired in cfdp1-/- embryonic hearts, implying its regulatory role also in this developmental process. Findings from both knockdown and knockout experiments showed that abrogation of cfdp1 leads to downregulation of Wnt signaling in embryonic hearts during valve development but without affecting Notch activation in this process. We will present the phenotypic characterization of this mutant line.

P24

A SEC-MS workflow to identify changes in the cardiac protein complexome caused by the pathogenic R14del phospholamban mutation

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The R14del phospholamban mutation is associated with Arrhythmogenic cardiomyopathy (ACM) and dilative cardiomyopathy (DCM)1. On a cellular level, the R14del mutation is associated with calcium handling defects, ER protein-folding stress, aggregation, and metabolic changes. We recently demonstrated that PLN forms a large (>2MDa) Calcium Handling Super-Complex (CHSC) alongside SERCA2a and RyR2, and that this complex is disrupted in atrial fibrillation patients and PLN-KO mice2. We hypothesize that a similar disruption of the CHSC may contribute to the R14del PLN calcium-handling abnormalities. Disruption of further protein supercomplexes may contribute to other aspects of the R14del phenotype. Addressing these questions thus requires a combined exploratory and targeted complexome profiling approach.

Here, we present a streamlined workflow that facilitates rapid and reproducible complexome profiling from mouse tissue. Membranes were isolated from ventricular cardiomyocytes and solubilized in mild detergent3,4. Protein complexes were then fractionated by size-exclusion chromatography (SEC) and constituent proteins identified by data-independent mass-spectrometry. Protein complexes were identified and characterized using a novel custom statistical script in R (mCP-R).

Our technique was able to successfully reproduce the CHSC changes previously observed in PLN-KO mice. Furthermore, our optimized technique displayed a greater sensitivity, reproducibility and was able to detect protein complexes in a greater size range (up to 7500 KDa) than the established approach2. Taken together, we believe that this workflow offers a robust and versatile approach for the study of R14del cardiomyopathy and other cardiac conditions.

References:

1. PMID: 17010801
2. PMID: 31185731
3. PMID: 27148881
4. PMID: 11887205

P25

Fine-tuning the inflammatory response following cardiac injury to promote cardiac regeneration

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The pronounced inability of adult human heart to regenerate causes millions of deaths following cardiac insult. The extent and persistence of associated inflammation has been linked with adverse cardiac outcomes, including fibrosis and hypertrophy. However, characteristics of the inflammatory response including the maturity of resident macrophages or the activation status of infiltrating cells may differentially influence cardiomyocytes and cardiac fibroblasts, and thus regeneration. Unravelling crucial parameters of such interactions in appropriate biological systems should confer decisive intervention potential in a serious health problem.

In the past years we have studied in detail cellular and molecular players regulating pivotal events initiating or sustaining the progress to heart failure in mouse and zebrafish and we propose here to combine the systems to globally study these interactions. In particular, we are setting up a strategy for identification of factors secreted by innate immune cells that affect cardiac regeneration. Based on in vivo and ex vivo systems and using a cross-species platform we would assess the effect of differentially activated immunocytes on cardiomyocyte proliferation and evaluate their pro- or anti-regeneration potency.

P26

Prime editing to correct the LMNA Q493X mutation in human induced pluripotent stem cells.

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Background: Standard Heart Failure treatment most often fails to halt disease progression in patients with a mutation in the LMNA gene. The heritability of this disease provides a unique opportunity to use genome editing to correct the mutation and eventually cure the disease. In this study, we explored CRISPR/Cas9 and the recently developed CRISPR-based prime editing technology as genome editing tools to correct the LMNA Q493X mutation.

Methods and Results: CRISPR/Cas9 induces double-strand DNA breaks, which can be precisely corrected by the endogenous homology directed DNA-repair mechanism (HDR). We induced HDR to correct the LMNA Q493X mutation and determined precise editing in 150 human induced pluripotent stem cell (hiPSC) clones. Unfortunately, none of our clones contained the intended edit and passed all quality controls. We detected small insertions and deletions, karyotype instabilities, but even large deletions of several kb in length. Prime editing uses a catalytically impaired Cas9 endonuclease, which only induces a single-strand DNA break, fused to an engineered reverse transcriptase to replace a specific genomic region. We also used prime editing to correct the LMNA Q493X mutation and obtained 8 correctly edited clones. None of these clones contained a large deletion and no off-target site effects or karyotype instabilities were observed. In the edited clones we detected a re-expression of the corrected allele, which is lost in the Q493X hiPSCs due to non-sense mediated decay.

Conclusion: Prime editing is a highly efficient and precise technology which we successfully used to correct the LMNA Q493X mutation in hiPSCs.

P27

Human engineered cardiac tissues recapitulate the functional phenotype of a phospholamban mutation-related cardiomyopathy.

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Introduction: Heterozygous deletion of arginine 14 (R14del) is a mutation of the phospholamban gene (PLN) associated with dilated and arrhythmogenic cardiomyopathy. With no available cure, the development of representative disease models is critical for mechanistic and therapeutic studies.
Objective: To demonstrate the application of human engineered cardiac tissue (hECT) as a model of PLN-R14del cardiomyopathy.
Methods: We employed a library of human induced pluripotent stem cells (iPSCs) from patients with clinical cardiomyopathy who are PLNR-14del carriers, and from healthy family members without the mutation (non-carriers). Patient-specific cardiomyocytes derived from these iPSC lines were combined with collagen/Matrigel matrix to fabricate hECTs. Using custom integrated force sensors for optical post-deflection tracking, we performed functional studies with and without electrical stimulation to evaluate hECT contractility.
Results: Under spontaneous conditions, carrier-hECTs displayed significantly higher beat rate variability and lower contractile force, along with lower maximum rates of contraction and relaxation, compared to hECTs from non-carriers. During 1-Hz electrical field stimulation, this pattern remained, where carrier-hECTs displayed decreased contractility compared to hECTs from non-carriers. We also observed a trend towards longer relaxation time in the carrier-hECTs. These findings were confirmed using hECTs created with iPSC lines from two different families, under pacing frequencies from 0.5 to 3.0 Hz, and analysis time points from 1 to 4 weeks.
Conclusion: The hECTs from carrier cell lines recapitulated abnormal cardiac contractile function as observed in PLN-R14del patients. These hECT models will have future applicability to study disease mechanisms and test potential therapeutic interventions for PLN-R14del cardiomyopathy.

P28

The Effects of Different Types of Calorie Restriction on Transglutaminase 2 (TG2) in Aorta of Aging Mice

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Transglutaminase-2 (TG2) has been reported to have functions in aging-associated cardio-vascular-diseases (CVDs). Calorie restriction (CR) is one of the most efficacious interventions for CVDs. Aim of this study is to assess the effects of different types of CR on TG2 mRNA and protein expressions, and on TG2 targeting microRNAs at different mouse age.

Mice were divided into three groups at week 10 (baseline); Ad-libitum (AL), chronic calorie-restricted (CCR,15% CR), and intermittent calorie-restricted (ICR) in which 60% CR was applied for a week followed by three-weeks of AL-feeding in cyclic manner. Aorta and blood samples were collected at weeks-10,17/18 and, 49/50. TG2 protein and mRNA expression levels in aorta were measured by western blot and RT-qPCR. microRNAs were measured using GeneChip™ miRNA 4.1 Array in blood. TG2 targeting microRNAs were determined using miRNAtap package. Expression of corresponding microRNAs were measured in aorta.

As a result, expression of TG2 targeting miRNAs, miR-700-5p and miR-423- 5p, were significantly increased in ICR-R group compared to AL group at week-49/50 ($p<0.05$). Compared to week-17, TG2 protein expression levels were decreased by 27% and 52% in AL and CCR groups respectively at week-50, although this was not statistically significant. mRNA expression of TG2 in aorta is currently being analyzed.

In conclusion, miR-700-5p and miR-423-5p may play roles in the signaling regulation of TG2, which is an important enzyme in vascular aging, however more analyses are needed to draw better conclusion.

P29

Integrative single-cell analysis identifies developmental trajectories of cardiogenesis and noncoding mutations in congenital heart disease

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Congenital heart disease (CHD), the most common birth defects, is the clinical manifestation of anomalies in fetal heart development - a complex process involving dynamic spatiotemporal coordination among various precursor cell lineages. This complexity underlies the incomplete understanding of the genetic architecture of CHD. To define the multi-cellular epigenetic and transcriptional landscape of cardiac development, we generated single-cell chromatin accessibility maps of human embryonic heart tissues. These data identified eight major differentiation trajectories involving primary cardiac cell types, each associated with an array of continuous transcription factor (TF) activity signatures. This atlas allowed molecular comparison of the regulatory similarities and differences between iPSC-derived cardiac cell types with their in vivo counterparts. We leveraged deep learning models to construct cell type-resolved chromatin accessibility profiles from DNA sequence at base resolution, which were used to decipher underlying transcription factor motif syntax and predict the regulatory impact of noncoding variants observed in CHD trios. De novo variants predicted to affect chromatin accessibility in arterial endothelium clusters were significantly enriched in CHD cases vs controls with a 1.7-fold enrichment. We used CRISPR perturbations to validate three of the enhancers nominated as regulatory from these models, linking them to effects on the expression of JARID2, NFATC1, and TFAP2A. Together, this work defines the cis-regulatory sequence determinants of heart development and identifies disruption of cell type-specific regulatory elements as a component of the genetic etiology of CHD.

P30

Circadian and infradian rhythms of cardiac L-type Ca2+ channel transcription

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Diurnal variations in the ion channels that govern the cardiac excitation may provide the crucial link between circadian clock and cardiac arrhythmias, a leading cause of cardiovascular death, which are more likely to occur in the morning after waking. These have been mainly related to K+ channel circadian variations, but little is known about the the L-type calcium channels (LTCC), whose pore is formed by the Cav1.2 subunit.
Under normal light:dark cycle, we observed that Cav1.2 protein expression showed significant circadian variations in mouse ventricles, which peak at Zeitgeber time (ZT) 15. Coordinated oscillation of Cav1.2 mRNA level and its regulatory subunits is also observed with a peak at ZT12, along with classical oscillation of the actors of molecular clock machinery. Notably we noticed a clear relation between Cav1.2, CLOCK and REV-ERBα mRNA expression.
In vivo bioluminescence monitoring (BLI) of the Cav1.2 promoter activity using a transgenic male mouse model expressing luciferase under the control of the cardiac Cav1.2 promoter (PCaluc) show a significant oscillation in the Cav1.2 promoter activity with a 1.5-fold increase at ZT 9 and 18. A comparable circadian pattern of cardiac Cav1.2 promoter activity and gene transcripts was observed in isolated PCaluc ventricular cardiomyocytes and in cardiomyocytes derived from human induced pluripotent stem cells, after serum shock synchronization.
Collectively, our findings suggested that the cardiac LTCC is expressed rhythmically, peaking during rest to active period, in part under the intracardiac CLOCK-REV-ERb regulation, which might have an impact in cardiac physiopathology.

P31

Activation of nuclear receptor PPARβ/δ improves mitochondrial respiratory function during myocardial I/R

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Activation of nuclear receptor peroxisome proliferator activated receptor β/δ (PPARβ/δ) confers cardioprotection via pleiotropic effects including antioxidant and anti-inflammatory actions, resulting in amelioration of ischemia/reperfusion (I/R) injury; however, the underlying mechanisms are not yet fully elucidated. The aim of this study was to investigate the effect of PPARβ/δ activation on mitochondrial respiratory function. For this purpose, rats were treated with the PPARβ/δ agonist GW0742 and/or antagonist GSK0660 in vivo. Mitochondrial respiration and ROS production rates were determined using high resolution fluorespirometry. Activation of PPARβ/δ reduced mitochondrial stress during in vitro anoxia/reoxygenation and improved fatty acid oxidation (FAO)-dependent mitochondrial respiration rate and ROS production at oxidative phosphorylation (OXPHOS)-dependent state during ex vivo myocardial I/R. Determination of expression of components of FAO-linked respiration pathway and of transcription factors governing mitochondrial homeostasis showed that PPARβ/δ activation was accompanied by increased mRNA expression of carnitine palmitoyl transferase 1b and 2 (CPT-1b, CPT-2), electron transfer flavoprotein dehydrogenase (ETFDH), peroxisome proliferator activated receptor gamma co-activator 1 alpha (PGC-1α), nuclear respiratory factor 1 (NRF1) and succinate dehydrogenase A (SDHA). Increased activity of citrate synthase (CS) as well as preservation of cardiac ATP content post-I/R were also observed. In conclusion, activation of PPARβ/δ improves mitochondrial respiratory function during I/R through stimulation of both FAO-linked respiration and PGC-1α/ NRF1 signaling, resulting in cardioprotection.

P33

The desmin-deficient mouse: A model to reveal reverse cardio-oncology interplays?

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Heart failure and cancer share risk factors and likely molecular and cellular pathways. This potential interdependence is being reflected in recent analyses revealing higher mortality and incidence of cancer in pre-existing heart failure. Accordingly, animal models of cardiac injury have been employed both to confirm these notions and to investigate the underlying mechanisms. Inflammation and associated molecular players have been identified as common mediators, while cardiac remodeling appears to be a contributor to the severity of malignancy. The desmin-deficient mouse is a model of progressive heart failure owing to cardiomyocyte death and the associated spontaneous inflammatory response, enriched in macrophages and sustaining fibrosis. Importantly, this model accommodates the study of molecular and cellular interactions throughout the lifespan thus overcoming potential time-point selection biases. In addition, two crucial mediators of adverse cardiac remodeling in the desmin-deficient mouse, osteopontin and galectin-3, are also modifiers in malignancy. We hypothesized that the ongoing inflammation and remodeling events in the heart of the desmin-deficient mouse may promote carcinogenesis. Accordingly, we employed the Bl6.F10 melanoma cell engraftment method and we evaluated tumor progression. Compared to wt counterparts, desmin-deficient mice develop larger tumors with an altered composition of macrophage and T-cell subtypes. These preliminary experiments support a potential of the desmin-deficient mouse as a tool in the studies for reverse cardio-oncology.

P34

Revealing cellular interactions reflecting and modulating adverse cardiac remodeling with a genetic heart failure model

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Adverse remodeling events form part of the cardiac repair mechanisms but are commonly deranged promoting detrimental fibrosis and contribute to heart failure development. Non cardiomyocytes establish complex cellular interplays decisively affecting both cardiomyocyte homeostasis and fibrosis outcomes. Identification of molecular mediators and pathways affected are expected to allow the deployment of novel, more efficient, interventions to cope with the unresolved problem of defective cardiac repair. To globally study such interactions, we use an animal model of progressive fibrosis and heart failure development, the desmin-deficient mouse. In these mice a spontaneous inflammatory response, enriched in macrophages and associated with the mitochondrial-dependent cardiomyocyte death, triggers detrimental fibrosis sustaining heart failure. Using crosses with mice deficient in a series of important mediators we affect crucial parameters of cardiac cell types and monitor their outcome in vivo. Furthermore, we develop relevant ex vivo systems to unravel the cell interactions. According to such approaches we pursued macrophage-secreted galectin-3 that affects the repair abilities of cardiac fibroblasts and promotes cardiac dysfunction. On the other hand, preliminary data indicate that the deficiency of vimentin differentially affects repair activities both by cardiac fibroblasts and macrophages, while the NLRP3-inflammasome activation may affect not only the non-myocytes but also cardiomyocytes, partially mediating inflammatory responses.