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Abstract book

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Abstract no. 101

Logic messenger RNA for functional selection of human embryonic stem cell-derived cardiomyocytes

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Deriving cardiac populations from human embryonic stem cells (hESCs) for cell-based therapy has long been considered a promising strategy, though skepticism about its translation to practice has grown in recent years. Undesired cell heterogeneity has drawn much attention due to the tumorigenic potential as well as the cause for inconsistency in clinical performance. As a result, various methods have been proposed to enrich hESC-derived cardiomyocytes (hESC-CMs) and eliminate the residual tumorigenic potential. In this work, we propose an alternative approach that takes advantage of both the unique molecular apoptotic mechanism of hESCs and the transient expression of specific miRNAs during cardiac differentiation. We generated sequence-engineered synthetic miRNA-responsive lethal mRNA construct that selectively induces apoptosis only in desired cell populations. We show that the miRNA silencing mechanism is able to withstand transgenic expression of S184del mutant Bax (mBax) mRNA that is capable of inducing rapid apoptosis in hESCs within several hours. By inserting targets site for the cardiac abundant miR-499-5p along the 3' UTR of mBax mRNA, we enriched hESC-CMs and reduced their pluripotency as the percentage of cells expressing cardiac troponin increased, while of OCT 3/4 declined significantly. Furthermore, injection of hESC-CMs treated with our mRNA did not form tumors in mice as appose to untreated hESC-CMs. Physiological analysis showed that this treatment did not impair the contractility and intracellular Ca^{2+} response of early stage hESC-CMs as opposed to glucose depletion treatment that significantly increased the Ca^{2+} transient decline and the relaxation of contraction. These findings shed light on the potential use of such synthetic logic mRNA for the safe removal of selected populations after differentiation and can have an important implications for cell based therapy strategies.

Abstract no. 102

Eliminating contraction during culture maintains global and local Ca2+ dynamics in cultured rabbit pacemaker cells

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Pacemaker cells residing in the sinoatrial node generate the normal heartbeat. Ca^{2+} signaling controls the heart rate by affecting membrane molecules directly, and indirectly through activating of calmodulin-AC-cAMP-PKA signaling. Thus, the physiological role of signaling in pacemaker cells can only be assessed if the Ca^{2+} dynamics are within the physiological range. Cultured cells that can be genetically manipulated and/or virally infected with probes are required for this purpose. Because cultured rabbit pacemaker cells experience a decrease in their spontaneous action potential (AP) firing rate below the physiological range, Ca^{2+} dynamics are expected to be affected. However, Ca^{2+} dynamics in cultured pacemaker cells have yet to be reported. We aimed to develop a modified culture method that sustains global and local Ca^{2+} kinetics alongside the cell AP firing rate. We used experimental and computational tools to test the viability of cultured rabbit pacemaker cells by changing culture dish coating, pH, phosphorylation, and energy balance. The cells were maintained in culture for 48h using two types of culture media: one without a contraction uncoupler and one enriched with either BDM (2,3- Butanedione 2-monoxime) or blebbistatin. The uncoupler was washed out prior to the experiments. Cells were successfully infected with a GFP adenovirus cultured with either uncoupler. Using either uncoupler during culture led to the cell surface area being maintained at the same level as fresh cells. Moreover, the phospholamban and ryanodine receptor densities and their phosphorylation level remained intact in culture when either blebbistatin or BDM were present. Spontaneous AP firing rate, Ca^{2+} kinetics, and local Ca^{2+} release parameters were similar in the cultured cells with blebbistatin as in fresh cells, but were affected by BDM. We showed that by eliminating contraction, phosphorylation activity is preserved and energy is reduced. However, the side effects of BDM render it less effective than blebbistatin.

Abstract no. 103

Identifying the role of cAMP/ PKA signaling in the crosstalk between energetic and electrical activities in the atria

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cAMP/PKA signaling regulates atrial cell function by affecting the kinetics of membranal and intracellular molecules. Atrial cells have a high cAMP/PKA level due to Ca^{2+}-activated calmodulin. However, their spatial and temporal kinetics have not been quantified under physiological conditions. A culture method is essential to characterize atrial cAMP/PKA activity. Unfortunately, previous rabbit atrial cell culture studies were unable to maintain cell function. Therefore, any phosphorylation measurements were not physiologically relevant. Our study has two aims: (1) to establish a rabbit atrial cell culture method capable of maintaining cell function and ability to be electrically stimulated at a physiological rate (the physiological beating rate of rabbit atrial cells is 3 Hz) and (2) to characterize cAMP/PKA activity in different atrial cell compartments.

The essence of the atrial cell culture method is to use 2, 3-Butanedione monoxime (BDM), a myofilament contraction inhibitor, for 24 hr and wash it from the cells before the experiments. The cells maintained their morphology, their ability to contract under stimulation of 1-3 Hz, and their global and local Ca^{2+} release characteristics in comparison to freshly isolated cells. Quantification of atrial physiological PKA activity required electrically stimulating the cells at frequencies of 1-3 Hz. PKA activity was measured in three separate cellular compartments: the cytosol, the mitochondrial matrix, and the outer mitochondrial membrane (OMM). PKA activity was normalized to its maximum and minimum using PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) and PKA inhibitor H-89 dihydrochloride-hydrate, respectively. PKA activity was significantly different in electrically stimulated atrial cells than in quiescent ones. In all compartments, increased electrical stimulation frequency elevated PKA activity (for 3 Hz stimulation: 5.49±0.47 for the cytosol, 5.14±0.45 for the OMM, and 2.79±0.22 for the mitochondrial matrix; all increases were fold-increases from quiescent PKA activity). Higher relative increases were found in the cytosol and the OMM. Therefore, when modeling PKA activity, different compartment kinetics should be considered. In conclusion, PKA signaling has different spatial resolution that depends on the electrical stimulation rate and its signaling activity is crucial for maintaining atrial cell function.

Abstract no. 104

Coupled-clock mediated stress in healthy and dysfunctional heart pacemaker tissue

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Normal automaticity of the sinoatrial node (SAN) pacemaker cells is regulated by integrated functions within a system of two coupled clocks. Stress by activation of the β-adrenergic receptors of the SAN leads to an increased beating rate under normal conditions. However, acute SAN stress was associated with arrhythmias in patients with mutations in the cardiac ryanodine receptor (RyR2) or in calsequestrin 2 (Casq2). This is the case, for example, in Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). Irregularities in Ca^{2+} handling have been reported in these mutant SAN cells; it is not known, however, how changes in Ca^{2+} lead to pacemaker dysfunction in these cells.

We hypothesize that (i) mechanical overload has a similar effect on pacemaker function as β-adrenergic stimulation; (ii) stress by either method in (i) can modulate SAN function through coupled-clock mechanisms; and (iii) changes in phosphorylation cascades also play a role in coupled-clock mediated stress.

We developed an experimental system that stretches mice SAN tissue and measures in parallel the beating rate using optic flow techniques. Stretching healthy mice SAN tissue or using β-adrenergic stimulation by isoproterenol led to an increase in the beating rate. To understand the internal mechanisms that mediate between the stress and pacemaker dysfunction in mutant pacemaker cells, we developed a MATLAB computational model. In the case of CPVT, the model predicts a reduction in phosphorylation activity in parallel to the reduction in Ca^{2+} transient amplitude. These signaling cascades directly and indirectly affect the T-type Ca^{2+} current (I_{CaT}), sodium current (I_{Na}) and sodium-calcium exchanger current (I_{NCX}). Impairment in SAN automaticity is thus mediated by these cascades. The same channels are responsible for the stress-induced increase in beating rate in healthy pacemaker cells. In addition, the model showed that indirect drug perturbation and gene expression can partially reverse mutation-associated pacemaker dysfunction.

In conclusion, in mutant SAN cells, alterations in internal pacemaker mechanisms such as Ca^{2+} and phosphorylation signaling lead to rhythm disturbance in response to stress via the same coupled-clock mechanisms that operate in healthy SAN cells.

Abstract no. 105

Rapid atrial pacing promotes atrial fibrillation substrate in unanesthetized instrumented rats

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The self-perpetuating nature of atrial fibrillation (AF) has been a subject of intense research in large mammalian models exposed to rapid atrial pacing (RAP). Recently, rodents are increasingly used to gain insight into the pathophysiology of AF. However, little is known regarding the effects of RAP on the atria of rats and mice.

Using an implantable device for electrophysiological studies in unanesthetized rodents, we examined the effects of continuous RAP for at least four consecutive days on the developed AF substrate of rats and mice. Aggressive burst pacing did not induce AF at baseline in the large majority of rodents, but repeatedly induced AF episodes in rats exposed to RAP for more than two days. A microarray study of left atrial tissue from rats exposed to RAP for two days vs. control pacing identified 304 differentially expressed genes. Enrichment analysis and comparison with a dataset of atrial tissue from AF patients revealed indications of increased carbohydrate metabolism and changes in pathways that are thought to play critical roles in human AF, including TGF-beta and IL-6 signaling. Among 19 commonly affected genes in comparison with human AF, downregulation of FOXP1 and upregulation of the KCNK2 gene encoding the Kir2.1 potassium channel were conspicuous findings, suggesting NFAT activation. Further results included reduced expression of MIR-26 and MIR-101, which is in line with NFAT activation. Our results demonstrate electrophysiological evidence for AF promoting effects of RAP in rats and several molecular similarities between the effects of RAP in large and small mammalian models.

Abstract no. 106

The small molecule *Chicago Sky Blue* promotes heart repair following myocardial infarction in mice

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The adult mammalian heart regenerates poorly after injury and as a result, ischemic heart diseases are among the leading causes of death worldwide. Nevertheless, recovery of the injured heart could be augmented by distinct modulations of the immune response, fibrosis, or cardiomyocyte survival, proliferation and contraction properties. Here we aimed to identify small molecules that could serve as novel cardiac repair agents following myocardial infarction (MI). For this, we designed a high-throughput screening system for small molecules that induce postnatal day-8 cardiomyocyte proliferation *in vitro* and found the small molecule *Chicago Sky Blue 6B* (CSB). We next demonstrated that CSB treatment reduces scar size and improves cardiac function of adult mice post MI, but does not induces adult cardiomyocyte proliferation *in vivo*. Although initially identified through *in vitro* screening for cardiomyocyte proliferation, in the adult mouse heart CSB improves cardiac function by two different mechanisms: (1) inhibition of CaMKII, which improves cardiomyocyte contraction; (2) inhibition of neutrophil and macrophage activation which attenuates the acute inflammatory response thereby leading to reduced fibrosis. In summary, we identified CSB as a novel potential therapeutic agent that enhances cardiac repair and function by suppressing post-injury detrimental processes, with no evidence for cardiomyocyte renewal.

Abstract no. 107

Genome editing by CRISPR/Cas-9 to reprogram cardiac mesenchymal stromal cells

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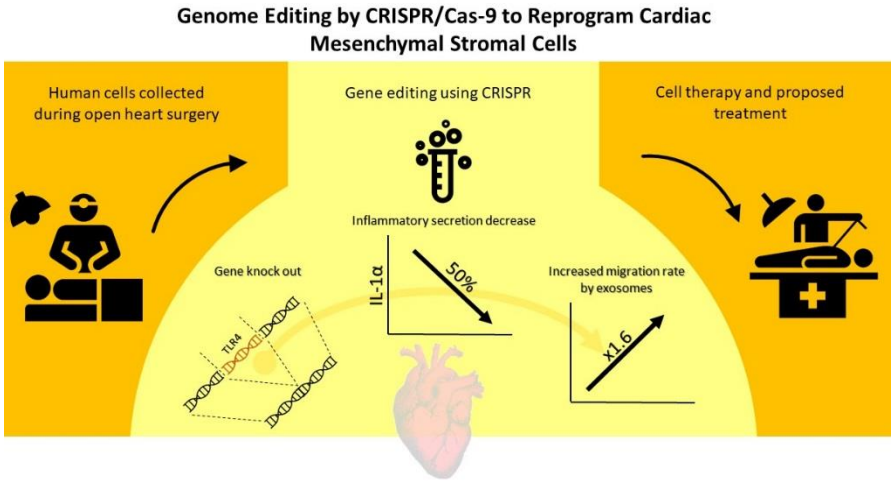
Background and Aim: The environment of the failing and infarcted myocardium drives resident and transplanted mesenchymal stromal cells (MSCs) toward a pro-inflammatory phenotype and restricts their survival and reparative effects in a mechanism mediated by the toll-like receptor 4 (TLR4). CRISPR/Cas9 is a promising tool for genome-editing DNA in cells with single-base-pair precision, which raises hopes for therapeutic genome editing in the clinic. We hypothesise that ex-vivo disruption of the human TLR4 gene by CRISPR -mediated genome-editing would switch the MSCs to an anti-inflammatory, reparative phenotype that could prevent remodeling of the left-ventricle post MI. **Methods and Results:** For gene editing, we electroporated a recombinant Cas9 nucleoprotein attached to a guide-RNA to create a double-strand DNA break at the desired location in the genome to induce a knock-out of the downstream gene. To evaluate the inflammatory response we tested secretome secretions from the cells. Our preliminary results show up to 63% success rate in editing the genome of the MSCs. In response, these cells were found to have significantly reduced inflammatory cytokine secretion, specifically of IL-1α ($p<0.00001$). Other cytokines with reduced secretion were IL-6, IL-15, IL-17, and IL-23 ($p=0.2000$ for all). Additionally, after finding a significant change in the exosome secretion profile from the cells ($p<0.0001$), we used exosomes to treat human-cardiac MSCs in a scratch assay. Exosomes from edited cells increased the fibroblast migration rate (83% scratch closure compared to 50%, after 24-hour incubation, $p<0.0001$). **Conclusion:** Our preliminary results suggest, for the first time, that human TLR4 gene-editing by CRISPR/Cas9 blocks inflammatory secretions and facilitates a reparative response by human-cardiac MSCs. The precise and efficient genome editing of TLR4 could provide a new strategy to improve MSC-based cell therapy to improve cardiac remodeling and function.



Abstract no. 108

Magnetically responsive scaffolds for multifarious stimulation of cardiac tissue patches

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Simulating the native environment of a cell in-vitro was, and still is, one of the most desirable achievements of tissue engineering. To succeed in doing so, one must provide external cues and stimulations to the cell culture to mimic the native niche of the cell. For example, the cells in the cardiac tissue experience a variety of stimulations - mechanical, electrical, chemical and other types. We used magnetically driven forces to create a cardiac culture platform that offers multifarious stimulation to cultured cells to simulate the cardiac cell niche.

By integrating alginate-coated iron-oxide magnetic nanoparticles (MNPs) into alginate scaffolds, and subjecting these scaffold to a permanent magnetic field during formation, the scaffolds acquired anisotropic, direction-dependent properties. The magnetic alignment introduced three distinct classes of anisotropy into the scaffold - surface topography, macrostructure and physical properties. Nano- and micro-scale topographical cues are recognized as major regulators of many aspects in cellular life such as growth, migration and phenotype. For example, the myocardium is composed of highly-aligned syncytia of cardiomyocytes, arranged in cardiac muscle fibers, interlaced with collagen fibers. This direction-dependent (anisotropic) structure leads to anisotropic conduction of cardiac excitation and the direction-dependent contractibility of the myocardium. SEM images of the surface of the scaffolds, revealed “ridge-and-trench” like topographic features. The extent of surface alignment, as featured by the density and size of the topographic features, was dependent on MNP concentration and MNP size. Analysis of the pore structure using micro-CT revealed that the pore directions in the aligned scaffolds were more narrowly distributed around a common angle, compared to those of the isotropic scaffolds, implying that the fabrication of the scaffold in a magnetic field caused the pores to align. By compressing the scaffolds and monitoring their stress-strain behavior we measured Young’s modulus that discovered increased stiffness in the direction of the alignment. Mouse myoblasts, cultured on magnetically aligned scaffolds, have oriented in the direction of the alignment to a greater extent than isotropic, non-aligned scaffolds.

After cell are introduced to the scaffold it retains its magnetic properties, and in the past our group showed that such scaffolds can undergo deformation (contraction) in an oscillating magnetic field. This makes our platform able of mimicking multifarious aspects of the cardiac niche – anisotropic topography and contraction-induced mechanical stimulation.

Abstract no. 109

Delivery of anti-periostin sirna by targeting nanoparticles to activated cardiac fibroblasts after myocardial infraction

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Introduction After acute myocardial infarction (MI), cardiomyocytes are replaced by secreted extracellular matrix (ECM) proteins produced by proliferating and activated fibroblasts. One of the key ECM proteins produced by the activated fibroblasts is periostin, known to be a major effector of fibrosis after injury. Periostin-null mice were shown to be more prone for left ventricular wall rupture after MI, but those who survived the initial insult exhibited better cardiac function with less fibrosis. This indicates that periostin is important for wound healing at the initial stage after MI, but its persistence may lead to fibrosis.

Hypothesis We hypothesized that targeting periostin at an appropriate time after MI could be an efficient strategy to control fibrosis after MI.

Methods and Results Using laser capture microdissection (LCM), we established the timeline of periostin gene expression after MI in the borer of the MI, and identified day 4 to 7 post MI as the peak days, showing more than 100 times fold increase of periostin mRNA compared to healthy mice. We then generated nanoparticles (NPs) encapsulating siRNA to silence periostin and with a targeting ligand, anti-periostin, on their surface. The addition of anti-periostin is to target the activated fibroblasts. The NPs exhibited average size of ~170-220nm and slightly anionic surface charge (~ -7 mV). XPS analysis demonstrated the presence of anti-periostin on NP surface. *In vitro*, activated adult cardiac fibroblasts, treated with the anti-periostin targeted NPs containing siRNA to periostin, presented 10-fold greater cellular uptake compared to NPs without targeting unit. Quantitative PCR results demonstrated a 5-fold decrease in periostin mRNA compared to non -treated activated fibroblasts. Ongoing *in-vivo* experiments aim to reveal the biodistribution of the NPs in mice after MI compared to healthy mice.

Conclusion Site-specific and timely delivery of periostin siRNA to activated fibroblasts presents a novel therapeutic strategy with a potential to treat MI with reduced fibrosis.

Abstract no. 110

Depressed β-adrenergic inotropic responsiveness and intracellular calcium handling abnormalities in Duchenne Muscular Dystrophy patients' induced pluripotent stem cell-derived cardiomyocytes

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Introduction: Duchenne Muscular Dystrophy (DMD) caused by mutations in the dystrophin gene, is an X-linked disease affecting male and rarely adult heterozygous females. DMD is characterized by progressive muscle degeneration and weakness, loss of ambulation and death by the late 20's or early 30's. **Hypothesis:** Our goal was to test the hypothesis that DMD patients' iPSC-derived cardiomyocytes (iPSC-CMs) exhibit functional abnormalities contributing to the *in vivo* cardiac pathology. **Methods:** Dystrophin-mutated iPSC-CMs were generated from male and female DMD patients. To test the hypothesis, $[Ca^{2+}]_i$ transients and contractions were recorded from control and DMD cardiomyocytes, using the IonOptix Calcium and Contractility system. To investigate the molecular mechanisms underlying Ca^{2+} handling in DMD iPSC-CMs, phosphorylation patterns in the CREB signaling pathway were detected using the CREB signaling phospho antibody array. **Results:** While in control cardiomyocytes isoproterenol caused a concentration-dependent positive inotropic and lusitropic effects, DMD iPSC-CMs displayed a markedly depressed response. To determine whether the reduced response was due to dysfunctional β-adrenergic cascade or impaired downstream elements mediating common positive inotropic interventions, we investigated the effect of elevating $[Ca^{2+}]_{out}$. Like isoproterenol, in healthy iPSC-CMs, elevated $[Ca^{2+}]_{out}$ caused positive inotropic and lusitropic effects, while DMD iPSC-CMs were unresponsive. Next, we tested the functionality of the SR (the downstream common denominator for inotropic interventions) by measuring caffeine-induced Ca^{2+} release. In healthy iPSC-CMs caffeine caused an abrupt increase in $[Ca^{2+}]_i$ followed by a gradual decline in $[Ca^{2+}]_i$ level. In contrast, DMD iPSC-CMs exhibited a reduced caffeine-induced Ca^{2+} signal amplitude and recovery time. To decipher the molecular mechanisms underlying the DMD cardiomyocytes dysfunction, we employed an enzyme-linked immunosorbent assay-based CREB antibody microarray to determine the phosphorylation patterns in the CREB signaling pathway. Briefly, in DMD male and female iPSC-CMs, MEK2 was more phosphorylated at Thr394 by at least 2-fold and PKC theta was less phosphorylated at Thr538 by by at least 2.5-fold respectively. **Conclusion:** DMD iPSC-CMs exhibit intracellular Ca^{2+} handling, mechanical and molecular abnormalities.

Abstract no. 111

Protein synthesis within the sarcomere

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Introduction: We have previously shown that mRNA of sarcomeric genes and protein translation are localized to the sarcomere z-discs. However, methods to image the sites of translation of specific proteins are currently lacking, and the sites of translation of specific sarcomeric genes is not known.

Aim: We aim to develop a new method to image the sites of translation of specific proteins and apply it to sarcomeric genes.

Mehtods: Our method is based on tagging a specific protein, and fluorescently labeling it in a pulse chase experiment, to differentiate between the pre-existing proteins from the proteins newly synthesized during the chase period. We used adenoviral transduction to express HaloTag-Tropomyosin1 fusion protein in neonatal rat cardiomyocytes. The HaloTag is an inactive hydrolase that covalently binds HaloTag-Ligands, which are conjugated to a fluorescent dye. During the Pulse, we add a first fluorescent HaloTag-Ligand to label all the existing fusion proteins. After washing the cells and a variable incubation period we added a second HaloTag ligand with a different fluorophore to label the new Halo-tagged proteins translated during the chase incubation period. By imaging the cells in both fluorophore channels, we can distinguish between the newly synthesized from the pre-existing proteins.

Results: Our results show translation of Tropomyosin1 on both sides of the Z-disc. This is concordant with our previous data in striated muscle and cardiomyocytes, showing that ribosomes are localized on both sides of the Z-disc.

Conclusions: We developed a new technique to study the site of translation of specific proteins and used it show that tropomyosin is translated on both sides of the sarcomere z-disc. We want to further investigate the translation localization of other sarcomeric genes.

Abstract no. 112

MicroRNAs in aortic valve disease

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Aim & background: MicroRNAs (miRNAs) control gene expression at the post-transcriptional level and are associated with various cardiovascular diseases, including valvular heart diseases (VHDs). The aortic valve (AV) is the most commonly affected valve in VHDs. The objective of the study is to identify miRNA molecules expressed in VHDs and the differential expression patterns of miRNA in AVs with either calcification or rheumatism etiologies.

Methods: Human AVs were collected during valve replacement surgery. MiRNA molecules were extracted using a protocol adjusted for aortic valve tissue. The miRNA libraries were prepared and sequenced using the Next Generation Sequencing (NGS) approach. Identified miRNAs were validated by quantitative real-time PCR (qPCR). The receiver operating characteristic (ROC) curve analysis was performed to examine the ability of relevant miRNA to differentiate between calcification and rheumatism etiologies.

Results: Twenty four AV samples, 5 rheumatic and 19 calcified, were prepared for the NGS and successfully sequenced. Based on the NGS analysis, we identified, for the first time, more than 1000 miRNAs expressed in aortic valve tissue. The expression of miRNAs was validated by qPCR approach in 46 AVs, 13 rheumatic and 33 calcified, confirming that miR-145-5p, miR-199a-5p and miR-5701 were significantly higher in rheumatic aortic valves as compared with calcified AVs. ROC curve analysis revealed that miR-145-5p has sensitivity of 76.92% and specificity of 94.12%, AUC=0.88 (P=0.0001), and miR-5701 has sensitivity of 84.62% and specificity of 76.47%, AUC=0.78 (P=0.0001), whereas miR-199a-5p has sensitivity of 84.62%, and specificity of 57.58%, AUC=0.73 (P=0.0083). The AUC was the highest in miR145-5p, suggesting a good capability in distinguishing between two VHD etiologies, while the AUCs of the two other miRNAs were 0.7-0.8 representing a moderate capability.

Conclusion: In this study, we documented differential miRNA expression between on AV disease etiologies. The miRNAs identified in this study advance our understanding of the mechanisms underlining aortic valve diseas

Abstract no. 113

Ubiquitin proteasome system role in diabetic induced Heart Failure

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Background: Ubiquitin-mediated proteolysis of cellular proteins plays a key role in the regulation of numerous cellular processes in the body; deviations in the Ubiquitin Proteasome System (UPS) function underlie the pathogenesis of various diseases.

We aimed to decipher the association between UPS function in the heart of Type 2 diabetes (T2D) patients and the development of Heart Failure (HF).

Methods: A T2D murine model of db/db knock out mice was established, and was further challenged with Angiotensin II (AngII) for HF induction. UPS role was studied in both the pre-diabetic phase (6-weeks-old) and the diabetic phase (12-weeks-old) of db/db mice using RNA Sequencing, qRT-PCR for chosen UPS genes and Western Blot analysis.

Results: The diabetic group was found to have a reduction of two 20S Proteasome Beta sub units: PSMB8 (β5i) and PSMB9 (β1i). In addition, a member of the Deubiquitinating enzymes proteases family (DUB) USP18 and its substrate Isg15 were also down-regulated. The DUB family members are responsible for Ubiquitin removal and recycling and thus have an important role in UPS regulation. Once the diabetic group was challenged with Ang II we found that a different set of genes were altered; an up-regulation in a DUB gene (Uchl1) and in an E3 ligase gene (Neurl1a) were detected. As E3 ligases were found to regulate cardiac metabolism in heart failure, the change detected is of high importance.

Conclusion: Our findings demonstrate that diabetic mice exhibit a unique UPS gene profile compared to non-diabetic mice. Furthermore, a different UPS gene profile is detected once the diabetic mice are challenged with AngII from the one seen when using saline. The detected genes are part of the E3 ligase and DUB families or components of the 20S proteasome; all are considered as vital for a well-functioning UPS. Future studies should investigate the ability of UPS modulators to prevent the development of HF.

Abstract no. 114

Long-term beneficial effects of combined treatment with cardiac steroids and AKT inhibitors following myocardial infarction in rats

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Congestive heart failure (CHF), is a clinical syndrome defined as the inability of the heart to supply blood to peripheral tissues, with the required volume of blood and oxygen to meet their metabolic exigency. Despite advances in therapy, morbidity and mortality due to CHF remain high. Cardiac steroids (CS) such as digoxin and ouabain increase the inotropy of the heart and are being used for the treatment of CHF and atrial fibrillation. However, their small therapeutic range limits their use. It is well established that Na^+, K^+-ATPase inhibition mediates CS-induced increase in heart contractility. Our recent findings using zebrafish and rat experimental models showed that CS were more potent in increasing the force of contraction of heart muscle when administered together with AKT inhibitors. The aim of the present study was to examine the beneficial effect of long-term (weeks) treatment with the CS digoxin and the AKT inhibitor miransertib (ARQ 092). Acute myocardial infarction in rats was induced by LAD-ligation, following which the rats were treated with digoxin (0.5 mg/kg/day), miransertib (1 mg/kg/day) or combined treatment with the two for 23 days. Heart function was evaluated by echocardiography. Our results show that the combined treatment with digoxin and miransertib significantly enhanced heart muscle contractility compared to treatment with digoxin alone. The beneficial effect of the combined treatment was evident at days 3 to 23 following LAD-ligation. None of the pharmacological treatments affected heart rate or the size of the heart scar tissue following LAD-ligation. In addition, exposure of rat cardiomyocytes to digoxin and miransertib did not cause an increase in cell death compared with that seen with miransertib or digoxin alone. The results strengthen our notion that combined treatment with CS and AKT inhibitor increases the therapeutic index for CS and should spur the development of such a formulation for the treatment of CHF.

Abstract no. 115

Ramipril attenuates cardiomyopathy in Danon Disease

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Background: Danon disease (DD) is an X-linked disease that causes cardiomyopathy manifested by ventricular hypertrophy, myocardial fibrosis and congestive heart failure. DD arises by mutations in Lysosome associated membrane protein 2 (LAMP2) gene causing loss of protein function, a defect in autophagy and accumulation of autophagosomes in cardiomyocytes. We have previously characterized mice carrying a "human" LAMP2$^{\Delta 6}$ mutation. Male mutant mice initially develop mild cardiac hypertrophy followed by progressive fibrosis and reduced systolic function. The cardiac phenotype in DD mice is aggravated by angiotensin. In this study we examined the effect of inhibiting the renin-angiotensin-aldosterone (RAAS) and of the sympathetic system on the cardiac phenotype in DD.

Methods: DD and wild type (WT) mice (12-14 weeks age) were treated till age of 30 weeks with Ramipril (10 mg/kg by gavage) or Metoprolol (~100 mg/kg in drinking water). The effect of therapy was assessed by echocardiography, quantitation of fibrosis, assessing oxidative stress through Malondialdehyde (TBARS) and gene expression analysis.

Results: Treating DD mice with Ramipril prevented left ventricular hypertrophy (maximal wall thickness MWT 0.92±0.34mm vs 1.14±0.1 in untreated DD, $p<0.05$, and similar to 0.92±0.1 in WT mice). The fractional shortening improved to 31.0±14.5 vs 20.6±8.3% in untreated DD, $p<0.05$, and similar to 27.6±8.3% in WT mice). Quantitative analysis of fibrosis in Masson Trichrome stained sections showed a decrease by Ramipril (3.8±3.6% compared to 13.4±2.5% in untreated DD $p<0.05$, but less than 1% in WT). Hearts of Ramipril-treated DD mice showed a decrease in Malondialdehyde content (0.78±0.41 vs 2.01±0.64 µM in untreated DD, $p=0.04$) and reduced expression of pro-fibrotic (Collagen a1) and pro-inflammation (TGF-beta and TLR4) genes. Treating DD mice by Metoprolol had no effect on cardiomyopathy.

Conclusion: RAAS plays an important role in development of cardiomyopathy in DD. Until specific therapy becomes available, patients may be treated by an ACE inhibitor to attenuate cardiac hypertrophy and adverse remodeling.

Abstract no. 116

DOCK10 is vital for normal cardiac function under neurohormonal activation

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Background: Dock10 is a guanine nucleotide exchange factor of Cdc42 and Rac1. These Rho family kinases have important regulatory roles in the heart presumably by modulating the activity of downstream MAP kinases including p38 and JNK. Unpublished data from our group recently identified a possible link between a mutation in a highly conserved region of Dock10 and an autosomal recessive form of severe dilated cardiomyopathy.

Objective: we hypothesize that Dock10 serves as a critical regulator of cardiac signal transduction. In this study we characterized the functional and biochemical roles of Dock10 in the heart.

Methods: *In vivo*: mice homozygous for a Dock10 knock-out first allele (EM:04723) were compared to littermate controls by gravimetric analysis (heart weight to body weight ratio) and echocardiography at baseline and following two weeks of exposure to Angiotensin II (Ang II, 2 mg/kg/day). Baseline contractility and calcium handling of isolate cardiomyocytes were also evaluated using IonOptix measurements. Phosphorylation of p38 and JNK after acute injection of phenylephrine (PE, 10mg/kg) were evaluated by Western blots. *In-vitro*: Dock10 was ablated in neonatal mouse cardiomyocytes having floxed Dock10 alleles, using an adenovirus expressing Cre-recombinase (250 MOI). Six days following the ablation procedure cells were exposed to 50μM PE for 20 minutes before lysis.

Results: Mice with global Dock10 KO had normal body weight and cardiac size at three month of age. However, baseline echocardiography revealed reduced fractional shortening and IonOptix recordings demonstrated reduced contractility and elevated diastolic calcium in isolated KO cardiomyocytes. Following two weeks exposure to Ang II, Dock10 KO mice had increased heart weight to body weight ratio, elevated fibrosis and markedly reduced systolic function. In both in vivo and in vitro models, the baseline phosphorylation of p38 was elevated in the absence of Dock10 and the response of both p38 and JNK signals to PE exposure was attenuated.

Conclusions: Our data indicate that Dock10 is vital for normal cardiac signaling and function particularly under neurohormonal activation. Further work is needed to understand specific roles of Dock10 in the different cell types of the heart.

An implantable system for long-term assessment of atrial fibrillation substrate in freely-moving rats exposed to underlying pathological conditions

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The **Background:** Atrial fibrillation (AF) is a growing epidemic responsible for substantial economic costs, morbidity and mortality. Common pathological conditions such as heart failure, metabolic syndrome and increased activation on the renin-angiotensin-aldosterone system, converge to contribute to electrical and structural changes in the atrial tissue and promote AF by mechanisms that remain poorly understood. Drugs aimed to target atrial remodeling are attractive new options to prevent AF perpetuation. However, early pre-clinical testing of such drugs is currently difficult due to the absence of reliable, and affordable, animal models. In the present work we developed and validated a chronically implantable device, enabling serial evaluations of AF substrate in rats exposed to pathological insults. **Methods:** Rats implanted with our atrial pacing & recording devices were divided to three groups: a. Sham (n=10) subjected to solvent only and serving as a control, b. Aldo (n=9) subjected to excessive aldosterone levels c. MI (n=10) exposed to anterior wall myocardial infarction. AF substrate was evaluated two- and four-weeks post implantation using a standard AF induction protocol. Results were also compared to a previous Base group (n=12) in which AF substrate was studied one week following implantation only. All animals passed echocardiography. At the endpoint, left atrial histology was evaluated for fibrosis and serum samples were analyzed for inflammatory markers (IL-6, TNF-alpha). **Results:** Rats exposed to ALDO or MI demonstrated a progressive increase in atrial fibrosis and concomitant increase of AF substrate compared to the Base group. In the MI group, AF duration was correlated with infarct sized and inversely correlated with ejection fraction. Unexpectedly, the Sham group also developed marked AF substrate over time. Further analysis indicated that the later phenomenon was not related to atrial fibrosis or systemic inflammation. An additional experiment in which implanted rats could interact through a barrier also excluded chronic-social isolation as the cause for the AF substrate supporting the notion that local effects of the implanted electrode probably mediate this outcome. **Conclusions:** We demonstrate the first system for repetitive AF substrate evaluation in freely moving rats exposed to underlying pathological conditions. This system should greatly improve pathophysiology studying and reliable testing of new therapies.

Abstract no. 118

ErbB2-driven cardiac regeneration activates ERK-Yap-LINC mechanotransduction signaling resulting in EMT–like processes

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Cardiomyocyte (CM) loss due to ischemic injury results in adverse remodelling and fibrosis, inevitably leading to heart failure. We recently demonstrated in mice that temporal over-expression (OE) of activated ErbB2 (caErbB2) promotes cardiac regeneration by inducing robust and reversible CM dedifferentiation, proliferation and hypertrophy. Here we show that transient caErbB2 signalling promotes robust functional and structural heart regeneration when activated 3-4 weeks after myocardial infarction, when scarring is evident. RNAseq data suggested an EMT-like process driving heart regeneration in OE mice, supported by elevated CM motility and proliferation, cytoskeleton rearrangements, CM cell-cell separation, and ECM modulation. Focusing on molecular mediators, we identified YAP (the major effector of Hippo signalling pathway) as a downstream target of caErbB2. We show that Yap localizes to the nuclear membrane in association with members of the LINC complex (Linker of Nucleoskeleton and Cytoskeleton). Furthermore, we identified Hippo-independent phosphorylations on Yap at S352 and S274 to be highly enriched in OE hearts. pS274 Yap associates with Desmin intermediate filaments and peaks during mitosis. Viral overexpression of phospho-mutants dampened the proliferative competence of OE CMs, highlighting the importance of these Yap phosphorylation sites for mitosis. ERK inhibition blocked S274 phosphorylation, Yap transcriptional activity and cytoskeletal rearrangements resulting in sarcomere stabilization, and overall WT-like morphology. Taken together, we uncovered a novel ErbB2-ERK mediated, Hippo-independent mechnotransduction signalling that activates Yap S274 phosphorylation and downstream signalling, ultimately resulting in heart regenerative EMT-like response.

Abstract no. 119

Regulation of steroidogenic acute regulatory (StAR) protein and periostin by IL-1α: possible implications for recovery after myocardial infarction

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We recently discovered that StAR, a vital steroidogenic protein, is transiently and robustly expressed in non-myocytes of the left ventricular free wall (LV) after ligature of the left anterior coronary artery (MI) in mice (Anuka et al, Mol Endo 27:1502, 2013). The present study shows that StAR expression is confined to resident fibroblasts and endothelial cells, the two pivotal cell types responsible for the onset of the sterile inflammation after MI. Consistent with this notion is the finding that StAR expression is restricted to the inflammatory phase of the response to MI, and IL-1α released from necrotic myocytes at the site of the ischemic injury, is also the predominant inducer of StAR in primary cardiac fibroblasts. In the latter cell model, we now show that incubation with IL-1α prior to treatment of the cells with cisplatin, protects the cardiac fibroblasts against apoptosis. In this respect, StAR is probably a necessary player downstream of IL-1α signaling since the anti-apoptotic resilience to cisplatin was found proportional to the level of StAR expression and ablated by use siRNA.

The relevance of IL-1α signaling for StAR expression was demonstrated *in vivo* by use of global IL-1α deficient (K/O) mice. Experimental MI in such animals resulted in more than 90% reduction of cardiac StAR expression, suggesting that IL-1α is indeed the predominant inducer of StAR. Interestingly, loss of StAR also associated with 70% reduction of periostin, known as marker for tissue repairing activated fibroblasts. Loss of StAR and periostin expression in primary cardiac fibroblasts treated with specific inhibitors of IL-1α signaling was consistent with the role of this cytokine in StAR/periostin expression.

In sum, our current working hypothesis assumes that StAR is a 'new player' in wound healing after MI. We suggest that while IL-1α ignites the inflammatory response to the ischemic injury of MI, it also induces StAR expression that protects the fibroblasts through the pro-apoptotic inflammation arena infiltrated by active phagocytic neutrophils and monocytes/macrophages. Their survival allows the fibroblasts to proliferate and differentiate into mature myofibroblasts responsible for repair of the damaged tissue and prevents rapture of the LV free wall.

Abstract no. 120

The tale of the tail: human induced pluripotent stem cell-derived cardiomyocytes vs. animal models for modeling human cardiac electrophysiology

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Pre-clinical and basic science research apply animal models to study various biological questions, such as drug safety and cellular biology. This kind of research focuses mainly on mammals, e.g., mice and rabbits. The similarities between these mammals and humans have contributed to medicine and science; however, we do not know to what extent these mammals are representative of human physiology, and particularly, human cardiac physiology.

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) serve as an additional, in-vitro model for cardiac cellular and electrophysiological studies. The use of this accessible source of cells, which are very similar but not identical to human cardiac cells, is increasing.

In this research, we employ electrophysiological recordings to assess the similarities and differences between cardiac electrophysiological signals from humans, hiPSC-CMs, and mammalian animal models. These recordings include an electrocardiogram (ECG) for a whole mammal or an electrogram for an ex-vivo or in-vitro sample, which are common methodologies in cardiac physiology research.

We analyzed six different datasets: human, rabbit and mouse ECG recordings; rabbit and mouse ex-vivo sinoatrial node tissue electrogram recordings; and spontaneously beating cardiomyocyte tissue derived from hiPSC-CMs. We compared the signal properties of all non-human datasets to human ECG data.

To perform this comparison, we analyzed the interval variations between consecutive beats of the ECG and electrogram recordings, known as heart rate variability. Additionally, we studied the complexity hidden in each beat interval signal as a mathematical function. The typical interval duration (and beating rate) is obviously different for different mammals, but the signal complexity is not necessarily different. Indeed, we found significant differences in rabbit and mouse ECGs and sinoatrial node electrograms as compared to human ECG data. However, signal complexity for hiPSC-CM tissues was found to be more similar to that of human ECG recordings.

These findings indicate that the choice of biological model for an experiment is not always trivial and depends on the research goal. hiPSC-CMs are a more compatible model than rabbits and mice for human cardiac electrophysiological research and may contribute to better clinical implementations of laboratory discoveries.

Abstract no. 121

Distinctive inflammatory and fibrotic signature of extracellular vesicles from epicardial fat of patients with Atrial Fibrillation

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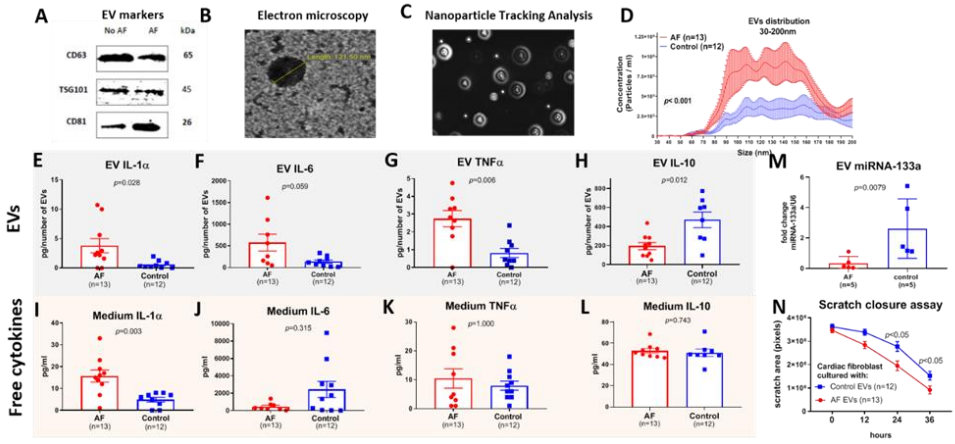
Background and Aim: Epicardial fat (eFat) has been linked to atrial remodeling and fibrillation (AF). We aimed to determine whether extracellular vesicles (EVs) derived from eFat play a role in the pathogenesis of AF.

Methods and Results: We collected small specimens of eFat from patients (pts) with and without (w/o) AF undergoing heart surgery. eFat specimens were incubated as organ cultures and EVs were isolated from the culture medium by ultra-centrifugation. We used immunoblotting, electron microscopy, and nanoparticle tracking analysis to characterize the EVs (**Fig. A-C**). Significantly, eFat specimens from AF pts secreted greater amounts of EVs compared with patients w/o AF (**Fig. D**). Moreover, eFat EVs from AF pts secreted a higher concentration of inflammatory and fibrotic cytokines but less anti-inflammatory cytokines, compared with patients w/o AF (**Fig. E-H**). Notably, EV cytokines reflected the inflammatory and fibrotic status of eFat in AF pts better than free cytokines (**Fig. I-L**). Next, we tested several miRNA that could influence cardiac fibrosis. For example, miR-133 inhibits TGF-β, decreases collagen content and inhibits atrial remodeling. Expression of EV miR-133 was lower in eFat of AF pts than in patients w/o AF (**Fig. M**). Eventually, "wound healing" scratch assay show that fibroblast migration was greater after incubation with eFat EVs from AF patients, compared with pts w/o AF. (**Fig. N**).

Conclusions: eFat from AF patients secretes a higher number of EVs with an inflammatory and fibrotic profile. Our findings suggest that eFat EVs contribute to inflammation and fibrosis, both of which contribute to the pathogenesis of atrial remodeling and fibrillation.



Abstract no. 122

Functional study of enhancers

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Introduction: The multitude of different cell types and organs in the body acquire different morphologies and functions by expressing different sets of genes. Accordingly, the different cell types in the heart, such as the cardiomyocytes (CM) and the cardiac fibroblasts (CF) express both shared and distinct sets of genes. Studied suggest that the regulation of tissue specific genes mostly depends on distal regulatory elements called enhancers. However, the laws governing enhancer activity on nearby gene expression is still unclear.

Aim: we hypothesize that recruitment of a strong activation domain to any area in the genome (CM and CF-specific enhancers and non-enhancer regions) would result in formation of enhancers, with recruitment of co-factors, and that this synthetic 'transcription factor' will be able to modulate the expression of multiple genes within the locus, in a manner dependent on distance from the nearby gene.

Methods: To test our hypothesis, we used a synthetic approach and targeted a catalytically dead dCas9 complexed tethered to strong activation domains in rat fibroblasts, to numerous target genomic points encompassing around 100kb bases around the start site of the gene of interest. Both fibroblast-specific enhancers and non-enhancer regions were targeted. We measured subsequent gene activation by means of RT qPCR.

Results: By targeting large areas around a gene we showed how a synthetic strong 'transcription factor' can activate cardiac specific genes in fibroblasts from distances of up to 70 Kb from the gene. We characterized the distance dependent effects as well as the activation domain effects of these synthetic enhancers. We further show that the induced synthetic enhancers and the promotor of the corresponding genes, acquire active enhancer specific epigenetic marks.

Conclusions: Our data sheds light on the rules governing enhancers -gene functional interactions, and the role of TF binding in the formation and subsequent activity of enhancers.

Abstract no. 123

Non-invasive thermal imaging identifies left ventricular remodeling in mice

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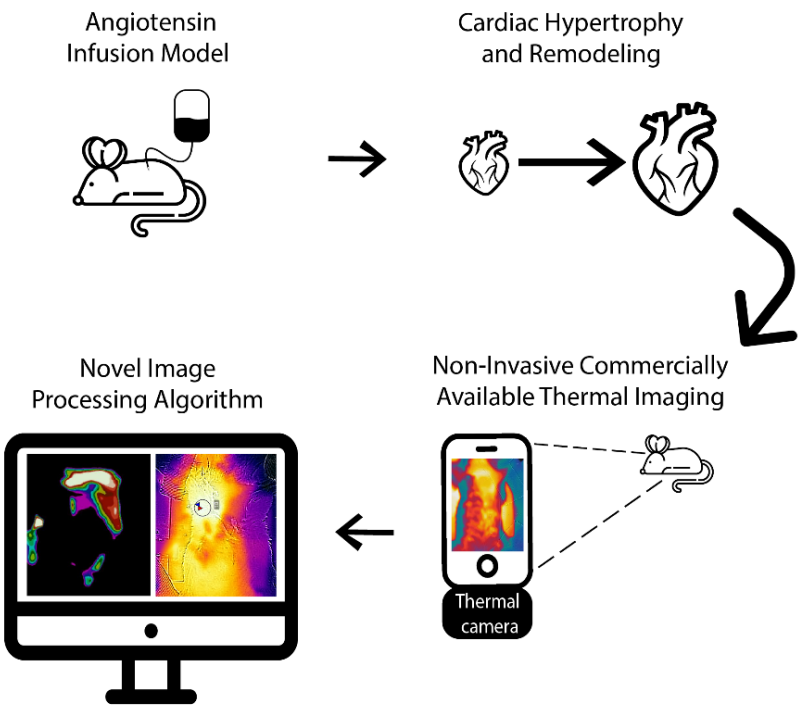
Background: Thermal infrared imaging is a non-invasive tool with the potential to screen physiological processes and diseases. The use of this technique to image internal organs, such as the heart, has not yet been investigated. We aimed to determine the ability of a novel algorithm for thermal image-processing to detect structural and functional changes in a mouse model of cardiac remodeling.

Methods: We randomized 12 male mice (weight 20-25 gr) to treatment with either angiotensin-II (2 mg/kg/day, n=6) or saline (n=6) pump-infusion for 28 days. We measured blood pressure weekly, together with serial trans-thoracic echocardiography studies and histopathological evaluation of the hearts. We captured thermal images with the commercially available FLIR One camera, and processed images by our novel algorithm.

Results: Angiotensin infusion increased blood pressure together with cardiac hypertrophy and fibrosis. Thermal imaging identified an increase in the fraction of the skin heated by the heart in angiotensin-treated mice, at day 28 of the experiment. Thermal image findings were correlated to left ventricular mass and volume by echocardiography ($r= 0.6$, $p=0.07$ and $r=0.8$, $p< 0.01$). By thermal imaging, all angiotensin-treated hearts displayed a unique triangle-like shape of heat distribution. This finding was absent in controls, indicating remodeling in the hypertensive heart.

Conclusion: Our preliminary findings suggest, for the first time, that a thermal camera with a new image-processing algorithm, identifies cardiac structural changes in mice. Our findings propose a new, simple, and non-invasive tool to diagnose and monitor adverse cardiac remodeling.


Angiotensin
Infusion Model
Cardiac Hypertrophy
and Remodeling
Non-Invasive Commercially
Available Thermal Imaging
Thermal
camera
Novel Image
Processing Algorithm
Accessible Non-Invasive Tool for Imaging Structural
Changes and Inflamation in Diseased Hearts

Abstract no. 124

Metabolic impairments in Duchenne Muscular Dystrophy (DMD): Searching for novel therapeutic approaches using patients' iPSC-derived cardiomyocytes

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Introduction: DMD, an X-linked muscle degenerative fatal disease is caused by dystrophin mutations. Dilated cardiomyopathy (DCM) is a major cause of morbidity and mortality in DMD patients. Treatments for DCM are still limited to standard adult heart failure medications and cardiac defibrillator implantation. To improve our understanding of DMD and discover novel therapeutic approaches, new strategies are needed. **Hypothesis:** We hypothesized that dystrophin gene mutations in DMD cause bioenergetic/metabolic deficits, thereby causing cardiac dysfunction. **Methods:** We generated induced Pluripotent Stem Cell-derived cardiomyocytes (iPSC-CMs) from male and female healthy volunteers and DMD patients. We investigated the bioenergetic and metabolic features of healthy and DMD iPSC-CMs using the Seahorse Flux analyzer and Liquid chromatography mass spectrometry (LC-MS) technologies. **Results:** The bioenergetic profile demonstrated impairments in glycolysis and oxidative phosphorylation pathways in both male and female DMD iPSC-CMs compared to healthy iPSC-CMs. We found a significant decrease of 75% and 70% in ATP production in male and female DMD iPSC-CMs, respectively, compared to healthy iPSC-CMs. Accordingly, most Krebs cycle metabolite levels decreased in female DMD iPSC-CMs compared to healthy iPSC-CMs. For example, α-ketoglutarate, cis-aconitate and citrate levels were 85%, 60% and 50%, respectively, lower in female DMD iPSC-CMs compared to healthy iPSC-CMs. Furthermore, in female DMD iPSC-CMs, there was a dramatic fall to undetected levels in phosphocreatine, along with high levels of creatine (up to 20-fold) compared to healthy iPSC-CMs. These findings indicate a dysfunctional phosphocreatine energy system. Moreover, energy source levels such as glucose, fatty acids and amino acids increased (up to 5-fold) in female DMD iPSC-CMs compared to healthy iPSC-CMs. **Summary and Conclusion:** DMD iPSC-CMs exhibit metabolic deficits and reduced ATP production. Dystrophin mutations in DMD adversely affect the biochemical pathways in iPSC-CMs and cause bioenergetic/metabolic imbalance.

Abstract no. 125

Continuous atrial fibrillation detection with physiological insights

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Atrial fibrillation (AF) is the most common cardiac arrhythmia in the Western world. AF has been found to greatly increase the risk of stroke, heart attack, dementia and overall morbidity. Because AF is mostly asymptomatic, most patients who suffer from this arrhythmia discover it only after a major clinical event, such as a stroke or heart attack. Our goal in this work is to detect the onset of AF events in real time, using mobile monitoring devices, to be used by in the general population. We used a gold-standard database from PhysioNet, which includes 3,543,396 normal sinus rhythm (NSR) beats, 5,245,274 AF beats and 266,966 beats labeled as other arrhythmia. We introduce a deep learning (DL) AF detection system, based on a time convolutional network (TCN). The input for the system is a raw ECG signal, from which we estimate the spectrum and auto-correlation. The raw ECG signal, spectrum and auto-correlation are all combined into a single input to the DL model. Our system detected the onset of AF events with an accuracy of 86.82 ±0.64% and precision of 92.66 ±1.1%, which surpass the previous state-of-the-art by 4.82%. We also performed ablation studies in order to determine the minimal sequence length of ECG recordings in [s] and the optimal number of ECG leads. We found that one ECG lead is sufficient for the detection of AF, and that a recording length of 16[s] yields optimal accuracy. However, for the optimal specificity, a sequence length of 32[s] should be used. Analysis of the DL model shows that, in the ECG channel, the expected location of P-Waves is accorded the greatest significance, in accordance with physiological expectation. Interestingly, comparison of the power-spectrums of AF and NSR samples, although generally similar, reveals that different harmonics play a role in the classification of a sample as normal, or arrhythmogenic. In conclusion, we report on a new method for the classification of ECG sequences as normal, containing AF events, or containing other arrhythmogenic events. Insights into the deep learning model also provide a new clinical index for the characterization of AF events.

Abstract no. 126

A possible non catalytic regulation of cardiac L-type Ca^{+2} channel by protein kinase A

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L-type voltage dependent Ca^{+2} channels ($Ca_V1.2$) are crucial in physiological regulation of cardiac excitation-contraction coupling. Ca^{+2} entry via $Ca_V1.2$ is up-regulated by activation of G protein-coupled receptors and protein kinase A (PKA). $Ca_V1.2$ regulation by PKA was attributed to phosphorylation of specific residues in the channel. However, hitherto, none of the PKA phosphorylation sites in $Ca_V1.2$ could be conclusively linked to the physiological cardiac effect in genetically engineered animals. Here we suggest an alternative mechanism for regulation of cardiac L-type Ca^{+2} channel by PKA. We found that the N-terminal (NT) cytosolic segment, which does not have phosphorylation sites, is involved in PKA-dependent up-regulation of $Ca_V1.2$. We demonstrated that mutagenesis of the first 4 amino acids or TYP motif in the NT diminished PKA up-regulation in Xenopus oocytes. This implies on non-catalytic regulation. Next, we co-immunoprecipitated PKA catalytic subunit (PKA-CS) with truncated $Ca_V1.2$ at position 1821 (Δ1821) and found that they directly interact. Pull down assays revealed that PKA-CS interacts with segments of the C and N termini. Mapping the interaction of PKA-CS in α1c C-terminus using peptide array revealed two main interaction sites: the proximal C-terminal regulatory domain (PCRD) and the distal C-terminal regulatory domain (DCRD). Point mutations in the PCRD, R1696-1697E and R1696-1697K led to decrease in binding in dot blot (RE and RK mutations, respectively). Two electrode voltage clamp Ba^{+2} current recordings in Xenopus oocytes expressing the RE and RK mutated α_{1C} showed analogues results, with small responses to cyclic AMP injection. These results indicate that α1c PKA-dependent up-regulation depends on non-phosphorylated residues, and that PKA-CS directly interacts with both the N- and C- termini. We conclude that at least part of $Ca_V1.2$ modulation by PKA-CS is mediated by direct binding to the channel.

Abstract no. 127

Atrial cardiomyocytes derived from human induced pluripotent stem cells for studying short QT syndrome related atrial fibrillation

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Background: Atrial fibrillation (AF) is the most common clinical arrhythmia and is associated with significant morbidity and mortality. Recent studies suggested that AF has strong genetic basis, including distinct monogenic cases of familial AF. Yet, the functional consequences of these variants on human atrial cells have not been fully elucidated due to the lack of appropriate human tissue models. One of the genetic causes is the short QT syndrome (SQTS), characterized by a short QT interval on the ECG and increased propensity for atrial and ventricular tachyarrhythmias.

Objectives: 1) To establish chamber-specific differentiation protocols to yield purified populations of human induced pluripotent stem cell (hiPSC)-derived atrial and ventricular cardiomyocytes (CMs). 2) To characterize the electrical properties of the SQTS-hiPSC-atrial CMs and to provide mechanistic insights into the atrial arrhythmogenecity in this syndrome**.**

Results: Patient-specific hiPSC lines from a healthy individual and a SQTS patient, carrying a missense mutation (N588K) in the hERG gene, along with a CRISPR/Cas9 corrected isogenic-control line were coaxed to differentiate to the cardiac lineage. Retinoic acid (RA) was either supplemented or not on days 3-5 to derive pure atrial or ventricular populations, respectively. Immunofluorescence of hiPSC-atrial CMs showed the absence of ventricular isoform MLC2v and upregulation of the atrial-specific TF COUP-TF1. FACS analysis further confirmed low percentages (<10%) of MLC2v+ populations. Patch-clamp recordings of the hiPSC-atrial CMs revealed shorter AP duration (APD_{90}: 233.2±1.5 vs 363.7±2.7 ms, $p<0.01$) and a more triangular APs with steeper repolarization (APD_{90}/APD_{50}: 3.07±0.41 vs 1.28±0.12, $p<0.05$) in comparison to APs from hiPSC-ventricular CMs. Voltage-clamp recordings confirmed the presence of the atrial ACh-sensitive potassium current (at -120mV: -3.8±1.65 vs 0.17±0.27 pA/pF, $p<0.0001$). Eventually, SQTS-hiPSC-atrial CMs exhibited even shorter AP duration compared to both healthy-control and SQTS-corrected-hiPSC-atrial CMs. (APD_{90}: 135.7±1.8 vs 233.2±1.5 and 204.3±1.6 ms, $p<0.0001$ and $p<0.05$, respectively).

Conclusions: 1) RA promotes the differentiation of hiPSCs to atrial CMs with distinct molecular and electrical characteristics. 2) hERG N588K mutation results in shortening of the AP duration in hiPSC-atrial CMs. 3) hiPSC-atrial CMs, coupled with gene editing technologies, offer a novel platform for studying the functional consequences of AF-related genetic variants

Abstract no. 128

Determining the active and passive contractile properties of single human pluripotent stem-cell derived cardiomyocyte at different preload conditions.

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Background: The advent of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) opened new avenues in physiological/pathophysiological studies and drug development. Current platforms used to assess the contractile properties of single hPSC-CM have inherent limitations; do not measure forces directly, require numerous assumptions, and most importantly, do not study the cell mechanics at different stretch levels. **Rational**: We aimed to establish a novel methodology that can overcome the aforementioned limitations by directly measuring forces (both active and passive) from single hPSC-CMs at different loading conditions. We then aim to evaluate the potential of this approach for different pathophysiological studies and for drug testing.

Methods and Results: Single hPSC-CMs were maturated using small molecules protocol, increasing their morphological and functional parameters. Single hPSC-CMs were attached by its two edges to two optical-fibers, one serves as a length-controller, allowing stretching the cell, and the other is connected to a highly-sensitive optical-force transducer that measure the forces at the nano-newton resolution. Active and passive properties were assessed by stretching the cell in a step-wise manner, hPSC-CMs exhibited positive length-tension relationship, with a quasi-exponential rise of the passive tension. Next we examined the effect of Isoproterenol (100nM) on hPSC-CM's contractility at 3 stretch levels. Isoproterenol application increased the force amplitude, the maximal contraction and relaxation velocities at all stretch levels. Finally, we evaluated the effect of the anti-neoplastic agent Doxorubicin (3μM) on the mechanical properties of these cells. Morphologically, Doxorubicin-treated hPSC-CMs displayed disrupted sarcomeres with reduced myofibrillar content. Doxorubicin-treated hPSC-CMs displayed markedly abnormal mechanics manifested by reduced active tensions and altered kinetics. Interestingly, we observed contractile alternans in 50% of the doxorubicin-treated hPSC-CMs. When investigating the force-frequency relationship, doxorubicin-treated group demonstrated exaggerated negative force-frequency compared to healthy hPSC-CMs. **Conclusion**: A novel method that allows direct active and passive force measurements from single hPSC-CMs at different loading conditions for the first time was established and validated. Our results highlight the potential implications of this novel approach for pharmacological studies and disease modeling; as we were able to identify drug-induced changes in the mechanical properties of the cells after isoproterenol stimulation and as a result of doxorubicin cardiotoxicity.

Abstract no. 129

The extracellular matrix protein Agrin promotes heart regeneration in mice

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The adult mammalian heart is non-regenerative due to the inability of cardiac muscle cells (CMs) to divide and produce new cells. In contrast, the neonatal mouse heart can regenerate through proliferation of existing CMs, but only for the first week of life. Extracellular matrix (ECM) has long been recognized as a modulator of cellular functions. In this work, we have employed a novel method to identify murine cardiac ECM compositions that promote CM proliferation and identified Agrin, a proteoglycan expressed by cardiac endothelial cells at birth with declining levels after 7 days. At birth, CMs from Agrin conditional knockout hearts (cKO) display mature and more differentiated phenotype accompanied with reduced proliferation and impaired cardiac regeneration. *In vitro*, recombinant Agrin promotes the division of human induced pluripotent stem cellderived and primary mouse CMs via a mechanism that involves the disassembly of the dystrophin glycoprotein complex and Yap and ERK-mediated signaling. *In vivo*, a single administration of Agrin promotes cardiac regeneration in adult mice after myocardial infarction by remodeling of the cardiac ECM as well as changing the immune landscape by modulating cytokine expression and macrophage recruitment. Furthermore, aspiring towards human therapeutics, recent set of experiments conducted in clinically relevant pig model of acute MI, have shown that delivery of Agrin has significant reparative effects in all parameters tested. These effects include reduction of infarct size, improved function and prevention of harmful remodeling. Collectively, we uncover a new inducer of mammalian heart regeneration, highlighting fundamental roles of the ECM in cardiac repair.

Abstract no. 130

Signatures of the autonomic nervous system and the heart's pacemaker-cells in an ECG record

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Heart rate variability (HRV) refers to the ever-present variations in the intervals between consecutive heartbeats. This phenomenon has been widely documented in mammals, including humans. The average heart rate and its variability are mainly determined by two mechanisms. The first is the autonomic nervous system (ANS) which interacts with receptors on the heart's primary pacemaker, the sinoatrial node (SAN). The ANS has two signaling pathways affecting the SAN cells: sympathetic activity, which increases the heart rate, and parasympathetic activity which decreases it. The second mechanism influencing HRV is the "coupled-clock" system within the SAN cells. This system is comprised of two intrinsic intercellular mechanisms ("clocks"), which interact with each other and are able to generate beats even without neural input.

Changes in heart rate variability are associated with cardiac diseases and timing of arrhythmogenic events. However, the relative contribution of the ANS and the SAN to changes in HRV are not clear, impeding effective treatment. We describe a strategy for discerning the contributions of ANS and SAN mechanisms to HRV changes, by analyzing standard ECG recordings.

We analyzed canine data which includes basal and ANS blockade segments (obtained by administering atropine and propranolol). To examine the SAN contribution, we applied various HRV analysis algorithms during both states. We analyzed the differences between these conditions to reveal the SAN and ANS contributions and define a set of features which represent the "signatures" of these systems. These features can be calculated from beat-interval time series obtained in-vivo, without using any pharmacological or invasive intervention. We validated our theory's relevance to humans on atrial fibrillation and heart failure patients as well as healthy aged subjects.

We found that the SAN and the ANS each contribute uniquely identifiable features to the long- and short-term variability of the heart rate: (a) the SAN regulates the heart rate with complex long-range variations, while (b) the ANS performs mainly short-term periodic regulation, which we modeled as sines embedded in white noise. We conclude that by applying HRV analysis, SAN and ANS function can be observed in regular ECG data. This may potentially enable future non-invasive diagnostic applications.

Computerized analysis of mammalian electrogram recordings

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Introduction: Electrograms (EGMs) are electrographic recordings of the surface voltage of a cardiac tissue. In particular, analysis of mammalian EGM of the sinoatrial node (SAN) tissue can be used to investigate the impairment of SAN functions in cardiac diseases. Today there is no tool that automates this analysis for different mammalian EGM. In this work we develop a beat detector algorithm to detect the position of beats and the beating intervals from EGM recordings obtained from different mammalian SAN tissue. This is a first tool toward computerized EGM analysis. It is implemented in the open source PhysioZoo software and available at physiozoo.com.

Methods: We collected data from 12 mice and 23 rabbits. SAN tissues were dissected from healthy young adults without any intervention and unipolar EGM recordings were performed. Overall, we recorded 106 minutes of EGM from rabbits and 75 minutes of EGM from mice. The beats were manually annotated using the PhysioZoo software interface in order to obtain the reference beats location. A total amount of 15,254 reference beats from rabbits and 20,331 reference beats from mice were obtained, totaling 35,585 reference annotations. The beat detection algorithm was implemented and performs the following steps: (1) preprocessing of the raw EGM, (2) naive peak detection by looking for local maximums, (3) rejection of peaks having width below a defined threshold, (4) rejection of peaks with amplitude below a computed threshold, and (5) beat to beat interval calculation. The performance of the beat detector was assessed against the reference manually annotated beats.

Results: The following average performance statistics and their standard deviations were obtained: Se = 0.995±0.014, PPV = 0.961±0.072, and F1 = 0.976±0.042 for the data from mice and Se = 0.977±0.061, PPV = 0.993±0.015, and F1 = 0.984±0.041 for the data from rabbits.

Conclusion: we created a novel beat detector for EGM recordings of different mammals (mouse and rabbit). The detector was implemented in PhysioZoo. It will serve to further investigate the beat-to-beat interval dynamics from healthy and pathological tissues.

Abstract no. 132

Resolving the Z-disc proteome using proximity labeling

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Objective: The assembly of protein complexes is essential to nearly all cellular processes. For these complexes to function, all components must be synthesized, delivered to the same subcellular location, folded and assembled in the correct order. Such assembly requires the localization of mRNA, ribosomes and translation factors to the complex. We hypothesize that sarcomeres are maintained by translation nano-domains that are localized on both sides of the sarcomere Z-discs. The molecular machinery required for this process and the mechanisms regulating transport and localization of its components to the sarcomeric Z-discs remains unknown.

Methods: To identify the molecular machinery that enables localized translation in the sarcomere we aimed to resolve the proteome of sarcomeric Z-discs. To this end we have performed proximity labeling using promiscuous biotin ligase tethered to the Z-discs of neonatal rat ventricular cardiomyocytes and used un-tethered biotin ligase as control. Labeled proteins were isolated and identified by mass-spectrometry.

Results: The z-disc proteome showed very high enrichment for ribosomal protein subunits, RNA-binding proteins, together with multiple known structural components of the Z-disc, consistent with an mRNA localization and translation nanodomain.

Conclusion: We identified the proteome of an mRNA localization and translation nano-domain at the sarcomere Z-disc. Further experiments are being performed to assess the role of individual Z-disc proteins in regulating these translation nano-domains.

Abstract no. 133

E-selectin-targeted copolymer reduces cardiac fibrosis in a mouse model of heart failure with preserved ejection fraction

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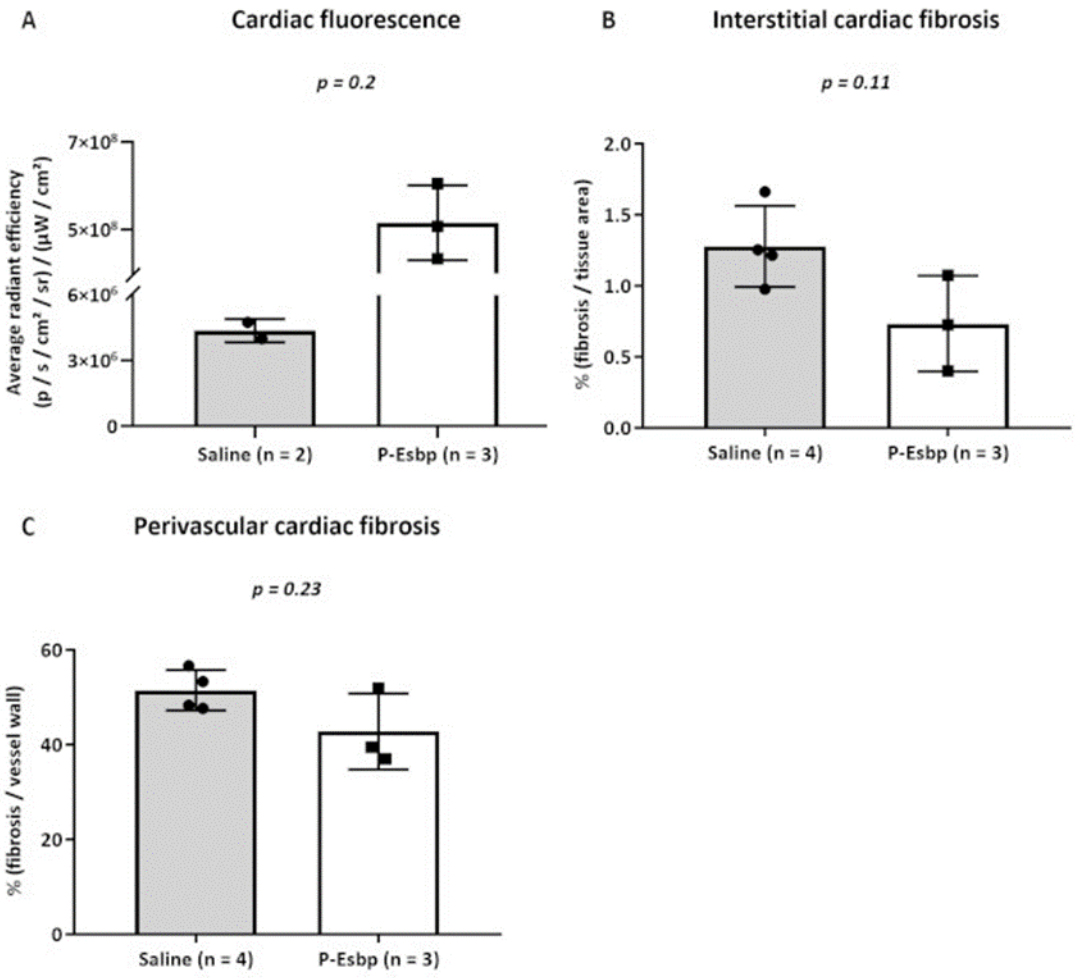
Background: Endothelial activation and dysfunction triggered by low-grade, systemic inflammation is a critical step in the pathogenesis of heart failure with preserved ejection fraction (HFpEF). In HFpEF, the adhesion molecule E-selectin is expressed on the activated endothelium and plays a key role in leukocyte rolling and migration, leading to inflammation, oxidative stress, myofibroblast activation and cardiac fibrosis.

Objective: To test the hypothesis that an E-selectin-targeted copolymer would attenuate cardiac fibrosis in a mouse model of HFpEF.

Methods and results: To induce HFpEF in 12-week-old male C57BL/6 mice, we implanted subcutaneous osmotic pumps for continuous infusion of angiotensin-II (2 mg / kg / d). To target E-selectin, we administered intraperitoneal injections of an N-(2-hydroxypropyl) methacrylamide-based E-selectin binding copolymer (P-Esbp) (0.5 mg / 0.2 ml), or saline, once a week for 4 weeks. Serial echocardiography studies revealed left ventricular hypertrophy with preserved ejection fraction in both groups. Ex-vivo imaging of whole hearts using an IVIS Lumina Series imaging system confirmed that near-infrared fluorescent-labeled P-Esbp targeted the diseased hearts (Fig. 1A). Microscopic examination confirmed that fluorescein isothiocyanate-labeled P-Esbp specifically targeted endothelial cells in the heart. Consequently, P-Esbp therapy reduced interstitial and perivascular cardiac fibrosis, as measured by post-mortem histology (Fig. 1B-C).

Conclusions: Our preliminary results show, for the first time, that an E-selectin-targeted copolymer reduces interstitial and perivascular cardiac fibrosis. Our findings confirm an important role for E-selectin and vascular inflammation in the pathogenesis of HFpEF, suggesting a new therapeutic target for the treatment of HFpEF.

Abstract no. 133



Abstract no. 134

Insights into healthy aged pacemaker function revealed by a longitudinal mouse study

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The U.S. Administration on Aging estimates that by the year 2050, 20% of the population will be 65 years and older. At the same time, chronic cardiovascular diseases, e.g., coronary artery atherosclerosis, hypertension and chronic heart failure, have reached epidemic proportions, with their occurrence increasing exponentially, along with risks of disability that increase with age. All these diseases are associated with changes in the heart rate and thus indicate pacemaker dysfunction. The heart pacemaker is a complex, sophisticated system controlled by the sinoatrial node (SAN) and the Autonomous Nervous System (ANS). These two coupled systems control the heart rate and its variability. Changes in heart rate variability (HRV) are associated with the risk of death. In this work, we investigate two questions: (i) Can we predict an age-associated increase in the risk of death only by exploring a set of statistical measurements of HRV? (ii) Which system—the SAN or the ANS—failed first and is thus associated with the increased risk?

To answer these questions, we examined ECG recordings of 59 healthy mice. Every three months (from the age of 6 months until the age of 30 months), an ECG was recorded for two hours under anesthesia under two conditions: basal, i.e. with no drug intervention, and intrinsic, by injection of atropine and propanolol to block, respectively, the cholinergic and β adrenergic receptors. We used the PhysioZoo platform for peak detection and HRV analysis. We analyzed HRV in three different domains: time, frequency, and non-linear features.

Using selected time-domain HRV features, we could predict which mice would survive past the 50% mortality age (21 months). The chosen features were PSS, the percentage of RR intervals in acceleration and deceleration segments with three or more RR intervals, and pNN5, the percentage of normal RR interval differences that are greater than 5 milliseconds. Using the frequency domain (very low frequency) and the non-linear domain (fractal behavior under long-term periodic regulation) makes it possible to separate between the effects of the ANS and SAN on HRV at different ages.

Thus, we propose a new way to predict age-related increase in the risk of death. We further claim that it is possible to detect whether the changes in the ANS or the SAN system are the main factor responsible for this increased risk.

Abstract no. 135

Verification of the cardiopulmonary vicious cycle paradigm in patients with dyspnea, a mechanism for accelerated decompensation

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Introduction: Dyspnea is the cardinal symptom of heart failure. We have shown that an increase in the respiratory effort (a surrogate of dyspnea) is associated with an increase in the pulmonary wedge pressure (PCWP), raising the question of cause and effect.

Aim: To investigate the immediate effects of the respiratory effort on the circulation by analyzing the responses to brisk changes in breathing patterns and to exercise.

Methods: The respiratory air flow and respiratory dynamics were measured in addition to the hemodynamics indices during right heart catheterization (RHC). The PCWP was decomposed into cardiac and respiratory waves. The respiratory effort (P_{RESP}) was defined as the amplitude of the respiratory wave (red arrow, left figure). The patients performed short events of apnea, vigorous breathing and physical exercise (0.5Kg weight lifting).

Results: The study enrolled 63 patients. The HF patients exhibited huge P_{RESP} of 9.6±3.7 mmHg, ~4 fold the normal P_{RESP} (2-3 mmHg). The PCWP rose with P_{RESP} by 0.92±0.36 mmHg for 1 mmHg of P_{RESP}. There was a bigger rise in PAP, due to the increase in the pulmonary vascular resistance (PVR). The PVR increased by 0.3±0.06 wood units for 1mmHg of P_{RESP}. Intriguingly, changes in the respiratory pattern and exercise had immediate (within a single breath cycle) effects on the PCWP (figure below). The minimum end-expiratory cardiac waves, denoted as P_{EEMin} (red dots), were immediately affected by P_{RESP}. A tight linear relationship was observed in each patient between P_{EEMin} and P_{RESP} (right figure). The brisk respiratory and exercise perturbations validate the immediate mechanical effects of the respiratory effort on the circulation. An increase in the negative intrathoracic pressure (P_{RESP}) increases the LV afterload, PCWP, PVR and the RV afterload.

Conclusions: The respiratory effort plays a pivotal role in the cardiopulmonary vicious cycle and has immediate detrimental effects on the PWCP and the afterloads of both ventricles.

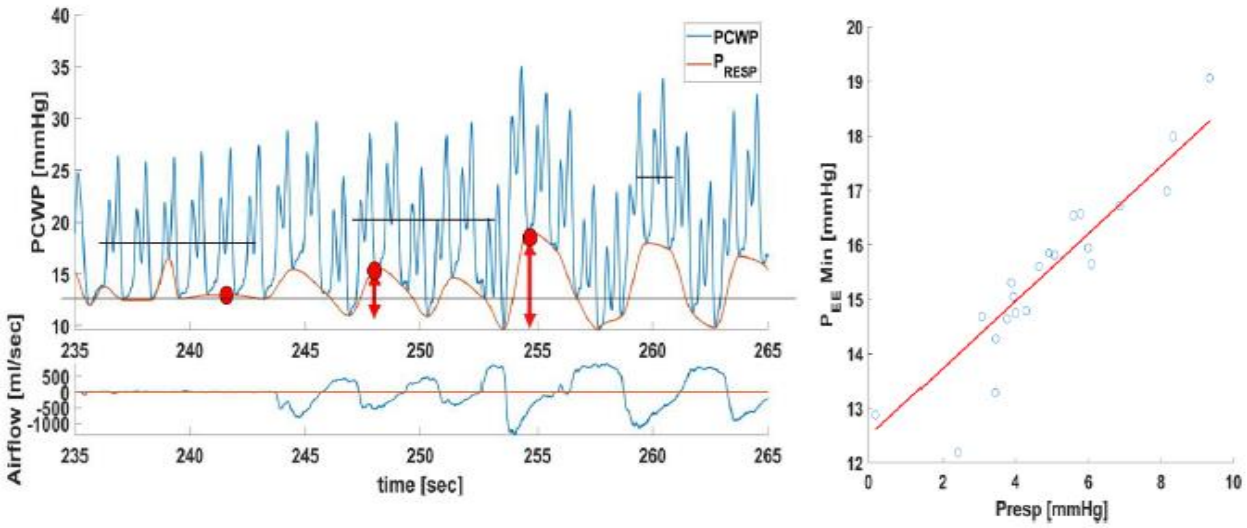

Figure. The respiratory effort (*PRESP*, arrows) immediately affects the pulmonary capillary wedge pressure (PCWP). A short apnea was followed by 2 normal breaths and vigorous breathing (airflow, left bottom). A linear relationship was obtained between *PRESP* and the minimum end-expiratory PCWP (*PEEMin*, right plot).

Cardiac relaxation is determined by velocity dependent transported cross-bridge cluster dynamics; Implications on Diastolic Dysfunction

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Background: Diastolic dysfunction causes heart failure in more than half of the heart failure patients. Current theories fail to explain how the relaxation rate is as fast the contraction rate, when cross-bridges (XB) detachment rate is significantly slower than XB attachment rate.

Methods: To precisely quantify the relaxation rate, a novel system for sarcomere length (SL) measurement was developed, with: spatial resolution of <2nm, temporal resolution of 150, and recording of SL distribution (inhomogeneity). Trabeculae were isolated from the rat right ventricles. The relaxation rate was studied at different initial preloads.

Results & Discussions: Empirically: relaxation was characterized by 3 phenomena: 1) Prolongation of contraction at higher loading conditions. 2) Parallel increase in the contraction and relaxation rates with the increase in the preload. 3) The point of maximum force relaxation rate appears simultaneously with the time point of maximum sarcomere lengthening rate, although sarcomere lengthening is generally associated with force enhancement.

Theoretically: Force generation was simulated by coupling calcium handling with XB dynamics. The recruited XBs have various energy levels. They are recruited at the highest energy level and the whole XB cluster is shifted/transported in time according to the rate of energy consumption. The energy depletion rate (transport rate) is a linear function of the velocity. Thus, a faster recruitment rate (contraction) is associated with a faster dissipation (relaxation) rate. The simulation successfully describes the above three characteristics of relaxation.

Conclusions: The novel theory, denoted as the “transported XB cluster theory”, provides explanations to the basic features of cardiac relaxation. The study provides better understanding of the sarcomeric control of relaxation, and how changes in calcium kinetics and XB cycling that determine cardiac contraction interact and affect the relaxation rate.

Abstract no. 137

Optogenetic Control of Action potential duration in human induced pluripotent stem cell derived cardiac tissue

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Aims: The morphology of the action potential (AP) in the heart is tightly regulated. any deviation in AP properties may increase the risk for arrhythmias. Here, we aimed to use optogenetic tools to control the AP morphology in in-vitro human multicellular cardiac tissue models and to correct abnormal AP duration (APD) in human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) established from patients with short and long QT syndromes (SQTs and LQTS), and to prevent the generation of reentrant arrhythmias.

Methods and Results: 293HEK cells were engineered to express the light-sensitive cationic channel CoChR or the light-sensitive anionic channel ACR2. The engineered cells were co-cultured with hiPSC-CMs to allow formation of gap-junctions and electrical coupling. Optical mapping was used to monitor the electrical activity of the co-cultures. Optogenetic stimulations were patterned at high spatiotemporal resolution using a digital micromirror device. The formation of reentrant in the hiPSC-derived cardiac cell sheet (hiPSC-CCS) model was induced by using a specialized optical cross-field stimulation protocol.

Light stimulation significantly prolonged APD in the cultures by 40%-100%. Moreover, APD prolongation led to a significant prolongation of the refractory period (RP) of the co-cultures by 40-100%. Next, we used our ability to modulate APD and RP to correct the electrophysiological properties in SQTS hiPSC-CCSs. By prolonging the APD optically within the propagating electrical signal we were able to decrease the rate of reentrant arrhythmia (spiral wave) generation. Illumination of the propagating signal with 26 pixels width light pattern decreased the number of arrhythmias by 26%, while illuminating with 66 pixels width light pattern prevent the generation of arrhythmias in 100% of cases. . In a similar manner we were also able to shorten the abnormal APD in LQTS hiPSC-CCSs by using light-activation of the ACR2

Conclusions: Optogenetics interventions can be used to control APD in human multicellular model. Such interventions allowed to prolong and shorten the tissue's action potential duration and refractory periods. The prolongation of APD in the SQTS model could be used to prevent the development of reentrant arrhythmias. These results highlight the potential of combining optogenetic and hiPSC technologies in providing mechanistic understanding of arrhythmogenicity and in designing future novel anti-arrhythmic therapies.