Session 8: Muscle in a dish: New models for the study of muscle function and dysfunction

Lectures

L8.1

Cardioids reveal self-organizing principles of human cardiogenesis

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Organoids capable of forming tissue-like structures have transformed our ability to model human development and disease. With the notable exception of the human heart, lineage-specific self-organizing organoids have been reported for all major organs. Here, we established self-organizing cardioids from human pluripotent stem cells that intrinsically specify, pattern, and morph into chamber-like structures containing a cavity. Cardioid complexity can be controlled by signaling that instructs the separation of cardiomyocyte and endothelial layers and by directing epicardial spreading, inward migration, and differentiation. We find that cavity morphogenesis is governed by a mesodermal WNT-BMP signaling axis and requires its target HAND1, a transcription factor linked to developmental heart chamber defects. Upon cryoinjury, cardioids initiated a cell type-dependent accumulation of extracellular matrix, an early hallmark of both regeneration and heart disease. Thus, human cardioids represent a powerful platform to mechanistically dissect self-organization, congenital heart defects and serve as a foundation for future translational research.

L8.2

Human-iPSC-derived three cell-type cardiac microtissues promote post-natal cardiomyocyte maturation and reveal contributions to heart disease

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Immaturity of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) is presently an obstacle in using them to model adult- or post-natal-onset cardiac diseases. In conventional two-dimensional cultures, hiPSC-CMs have fetal-like structural, functional, electrical, and metabolic features and express mainly fetal isoforms of developmentally regulated genes. We recently showed that tricellular combinations of hiPSC-derived CMs, cardiac fibroblasts, and cardiac endothelial cells enhance maturation in easily constructed, scaffold-free, three-dimensional cardiac microtissues (MTs). Using patient-derived hiPSCs, we show that cardiac fibroblasts can be active contributors to arrhythmia and that mature hiPSC-CMs express post-natal isoforms of cardiac ion channels, revealing mutation effects in heart disease. Our MT model is thus a simple and versatile platform for modelling multicellular cardiac diseases and for studying developmentally regulated cardiac genes. We will discuss directions and challenges in the field.

L8.3

Preclinical testing of personalized CRISPR treatments for Noonan syndrome

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Our understanding of the pathophysiological alterations and mechanisms in Noonan syndrome remains limited and effective therapeutic options are lacking. By generating induced pluripotent stem cell-derived cardiomyocytes from two affected siblings with biallelic variants in LZTR1, we were able to recapitulate the hypertrophic phenotype in vitro and uncovered a causal link between LZTR1 dysfunction, RAS–MAPK signaling hyperactivity, hypertrophic gene response and cellular hypertrophy. In a proof-of-concept approach, we explored a clinically translatable intronic CRISPR repair and demonstrated a rescue of the hypertrophic phenotype.

Oral Presentations

O8.1

The atrial cardiac myosin is a weaker force and power generator than the ventricular isoform as measured by the synthetic myosin nanomachine

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The functional differences of the α- and β-cardiac myosins that make up the atrial and ventricular chambers of the human heart have been described in detail with solution kinetic analysis, showing that α-cardiac myosin has faster ATP hydrolysis and faster ADP release so that the ATPase cycle time is ten times shorter, but the duty ratio (r) is similar to that of β-cardiac myosin. Instead, the isoform-dependence of mechanical performances remains elusive as the force of the β-cardiac myosin was found either twice higher (attributed to a larger r) with *in vitro* mechanics or similar in Ca^{2+}-activated skinned myocytes. Here, we use our synthetic nanomachine (Pertici *et al.*, 2018, *Nat Commun* **9**: 3532) to determine the performance of an array of 15 heavy-meromyosin fragments (HMM) purified from the bovine atrium and ventricle pulling on the actin filament in either isometric or isotonic condition. We find that α-HMM has an isometric force (6.6±0.6 pN) ~3-fold lower than the β-HMM (23.7±3.9 pN), and an unloaded shortening-velocity ~3-fold higher (1.4±0.2 and 0.5±0.1 μm s^{-1}). These mechanical parameters underpin a maximum power, an essential parameter for cardiac function, twofold lower for α- (0.7±0.1 aW) compared to β-HMM (1.4±0.1 aW). This quantitative description of the performances of cardiac myosin isoforms opens up the potential of the nanomachine for characterizing cardiomyopathy-causing myosin mutations and drugs currently developed for different therapeutic indications.

O8.2

Burst-like transcription of *MYH7* in Hypertrophic Cardiomyopathy patients is mimicked in patient-derived hPSC-CMs

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Transcription of *MYH7*-alleles occurs in stochastic bursts leading to unequal ratios of mRNA from both alleles among cardiomyocytes (CMs) from HCM-patients. This is associated with a functional heterogeneity among CMs, which could contribute to HCM-development. Here, we aim to establish a human pluripotent stem cell (hPSC-CMs)-based model for burst-like transcription in HCM. We tested whether transcription of *MYH7* also occurs in bursts and results in allelic imbalance from cell to cell in hPSC-CMs.

We performed RNA-fluorescence *in situ* hybridization (RNA-FISH) in hPSC-CMs with *MYH7*-mutation R723G to detect active transcription sites (aTS) in nuclei at different time points. We detected CMs with and without aTS at each time point, indicating burst-like transcription. Fractions of inactive nuclei were comparable in long cultivated (>34 days) hPSC-CMs (31%) and patient's CMs (27%). However, transcriptional activity was higher in patient's tissue (3.3 aTS/nucleus) than in hPSC-CMs (1.2 aTS/nucleus), presumably due to increased ploidy in patient's tissue. Nevertheless, allele-specific single cell RT-PCR from individual CMs isolated by laser microdissection revealed comparable highly variable allelic ratios among individual CMs in both hPSC-CMs and CMs from patient's tissue. Our results indicate that hPSC-CMs provide a suitable model to analyze and modulate transcription of *MYH7*-alleles to equalize heterogeneity among individual CMs as a potential therapeutic target in HCM.

O8.3

Modeling patient-specific Lmod2 dilated cardiomyopathy using human iPSC-derived cardiomyocytes

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Dilated cardiomyopathy (DCM) is associated with a high rate of mortality due to enlargement of the ventricular cavities in the heart, causing systolic dysfunction. Thus far a diverse group of genes have been recognized in the pathogenesis of DCM with mutations being identified in up to 40% of all DCM patients. Due to this high mutagenic rate a better understanding of these genetic mutations is needed. Recently, the first human Lmod2 mutation associated with DCM was discovered in a pediatric patient. Exome sequencing identified a homozygous nonsense mutation in the *LMOD2* gene. Leiomodin-2 (Lmod2) is a sarcomeric protein that has been shown to regulate thin filament lengths by acting as a nucleator of actin polymerization *in vitro*. In contrast, Tropomodulin-1 (Tmod1), functions to inhibit both actin polymerization/depolymerization from the pointed end of tropomyosin-decorated filaments. Together with Tmod1, Lmod2 precisely fine-tunes thin filament lengths and thus controls proper muscle contraction. To examine the effects of this *LMOD2* mutation we have implored the use of human iPSC-derived cardiomyocytes (hiPSC-CMs), as well as gene-corrected CRISPR/Cas9 isogenic controls. Using hiPSC-CMs we have examined alterations in the transcriptomic signature of the *LMOD2* mutation, studied structural changes at the level of the sarcomere and analyzed thin filament length regulation. Simultaneously, we have observed functional changes in mutant hiPSC-CM engineered heart tissues, which display altered contractile and calcium handling dynamics. Overall, the goal of this project is to decipher the pathophysiology underlying Lmod2 cardiac disease and to determine how an Lmod2 truncated protein can lead to such severe, early-onset DCM.

Virtual Posters

P8.1

In vitro modelling of Marfan related cardiomyopathy reveals abnormal behaviour of heart muscle cells

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Marfan syndrome (MFS) is a systemic disorder of connective tissue caused by pathogenic variants in the *FBN1* gene encoding for fibrillin-1, a structural component of microfibrils. Myocardial dysfunction has been demonstrated in MFS patients and mouse models but little is known about the intrinsic effect of the mutations on the heart muscle cells, the cardiomyocytes (CMs). CMs were obtained by differentiating healthy and MFS human induced pluripotent stem cells, establishing the first human *in vitro* model for MFS-related cardiomyopathy [1].

Several techniques were performed on the muscle cells, such as atomic force microscopy, multi-electrode array and Flexcell, revealing functional abnormalities of CMs. MFS CMs were stiffer, showed a lower beat-to-beat variability and received incomplete matrix support compared to corrected CMs. These results show that an impaired matrix plays a key role in the improper functioning of cardiomyocytes in MFS.

The MFS model has been further improved using 3D cocultures of CMs and cardiac fibroblasts (CFs) to understand the specific role of each cell type. Preliminary results show that abnormal matrix production of CFs plays a major role in the observed abnormalities of MFS CMs, while co-culturing with healthy CFs only shows partial rescue. We postulate that abnormal extracellular matrix of CMs may lead to MFS-related cardiomyopathy.

Refrences:

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P8.2

Transcriptome analyses of iPSC-derived skeletal muscles of isogenic Duchenne Muscular Dystrophy (DMD) lines at different differentiation stages

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Introduction: DMD is a severe x-linked muscular dystrophy affecting one in five thousand boys. DMD is caused by frame-shifting or truncating mutations in the *DMD* gene, leading to the complete absence of dystrophin in skeletal muscles. Deriving skeletal muscles from iPSCs is an emerging tool to explore cellular and molecular consequences of dystrophin absence. We sought differentially expressed genes (DE) in two DMD isogenic lines compared to the paternal wild-type NCRM1 line. DMD-1 lacks exon 52, and DMD-2 has a stop codon within the same exon. **Methods:** We performed RNAseq in triplicate samples from four differentiation stages and defined DE genes by comparing each line against NCRM1 (FDR $\leq 10^{-3}$ and FC threshold defined by power-law function). Hits present in both comparisons served as input for biological process and regulatory network analyzes. **Results:** We found 14, 779, 1810, and 910 commonly DE genes on the four stages chosen, respectively. Among the enriched pathways found are skeletal muscle development, muscle cell fate commitment, striated muscle adaptation, and muscle atrophy. **Conclusion:** We provide a benchmark of genes and pathways modified in iPSC-derived skeletal muscles of two DMD isogenic lines, which are helpful for future studies exploring muscular dystrophy in a dish.

P8.3

Pathomechanisms of human cardiomyopathy due to a titin truncation studied in hiPSC-derived cardiomyocytes

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A heterozygous truncation in TTN, which codes for the muscle protein titin, is the most common genetic cause of dilated cardiomyopathy (DCM). Pathogenicity is highest when the TTN truncating variant (TTNtv) is located in the constitutively expressed A-band region of titin. Here, we study the impact of a TTNtv and of pharmacological modulation of protein quality control (PQC) pathways on titin protein expression and contractility of human induced pluripotent stem-cell derived cardiomyocytes (hiPSC-CMs). TTNtv hiPSC-CMs were derived from a DCM patient with A-band-TTNtv or generated from a healthy hiPSC-CM line by CRISPR/Cas9 gene editing (M-band-TTNtv); wildtype (WT-)hiPSC-CMs served as controls. Ring-shaped engineered heart muscles (EHMs) were produced from patient-derived A-band TTNtv and WT hiPSC-CMs. PQC pathways were modulated by using autophagy-activator rapamycin, autophagy-inhibitor bafilomycin-A, proteasome inhibitors MG132 or bortezomib, and DMSO as the vehicle-only control. Both TTNtv-hiPSC-CMs and TTNtv-EHMs expressed less wildtype (wt-)titin protein than control cells, which was associated with contractile deficiency. The wt-titin protein content was restored by incubation with proteasome inhibitors, which reversed the contractile deficiency. Furthermore, we observed stable expression of truncated (tr-)titin proteins in TTNtv-hiPSC-CMs; their content increased upon proteasome-inhibition but not with autophagy-modulation. Genetic correction of the A-band-TTNtv in EHMs using CRISPR/Cas9 eliminated the tr-titin proteins and raised the wt-titin proteins to levels found in WT-controls, while contractility was fully rescued. Our findings suggest that the pathomechanisms of TTNtv-DCM include the accumulation of tr-titin proteins worsened by proteasome-inactivation, and the loss of wt-titin protein in TTNtv-CMs (titin haploinsufficiency).

P8.4

Impact of perinatal changes in the heart microenvironment on cardiomyocytes' proliferation: a study of miR15 family, hypoxia and energy source

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Adult cardiomyocytes (CMs) possess only a scarce regenerative potential, which is not enough in case of the deleterious event of myocardial infarction (MI). The ultimate treatment for acute MI is still heart transplantation, which is remarkably limited by the number of donor organs.
Embryonal development of the heart relies on extensive proliferation of the CMs. Shortly after birth, CMs exit the cell cycle. CMs stop dividing mainly due to increased oxygen levels and switch from glucose to fatty acids as energy sources. Thus, restoring the proliferative potential of the adult CMs by modulating oxygen level and energy source is a plausible idea.
The technology of the CMs derived from human pluripotent stem cells (hiPSC-CMs) provides an infinite source of the cells for investigation. A wealth of studies suggest that relatively short periods of severe (0.5-1% O_2) hypoxia might induce a CMs proliferation, basing on the expression of cell cycle-related genes. However, the field lacks knowledge of the long-term effects of physiological hypoxia on the expansion of CMs.
Our results show both HIF1α and HIF2α to be stabilized in long-term culture in 3% hypoxia. Additionally, the antiproliferative miR-15 family is downregulated in conditions mimicking the tension of O_2 in the developing heart microenvironment. Nonetheless, in our long-term study, these changes did not result in increased proliferation, as measured by expression of cell cycle-related genes and Ki-67 staining. Switching from glucose to palmitic acid as the primary energy source also failed to impact the proliferation of hiPSC-CMs in our study settings.
Our results indicate the need to understand the mechanisms of CM proliferation better to switch from prof-of-concept studies to the long-term expansion of the CMs.

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P8.5

Angiogenic potential of genetically engineered C2C12 myoblasts for muscle disease

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Angiogenesis is where new blood vessels form from pre-existing vessels. Adequate oxygen transport and waste removal are necessary for tissue homeostasis, restrictions in blood supply can lead to ischaemia which can contribute to disease pathology in muscle conditions such as atherosclerosis, peripheral artery disease or muscular dystrophy. Therapeutic angiogenesis is a potential treatment option with the aim of re-establishing blood flow. Vascular endothelial growth factor (VEGF) is essential in angiogenesis, while also seen to have a role in myogenesis, having been shown to encourage muscle repair following injury, making it an ideal candidate as an angiogenic and myogenic stimulant in muscle. **Objectives:** To establish a muscle cell line stably expressing VEGF-A and to determine its potency in eliciting an angiogenic effect in the chick chorioallantoic membrane (CAM) and endothelial cell models of angiogenesis. **Methodology:** C2C12 cells were stably transfected to express VEGF-GFP and GFP. ELISA determined VEGF-A secreted from stable cells into cell media while angiogenic assays were then used to investigate the angiogenic effect of these cells. **Findings:** We successfully have characterised VEGF-A expression and proven the angiogenic potential of stably transfected C2C12 mouse myoblast cells. VEGF-A stable transfected C2C12 cells secreted significantly increased amount of VEGF-A, compared to GFP transfected. Conditioned media from cells produced a significant increase in angiogenesis, while the cells themselves also elicited a strong angiogenic response directly. These studies qualify the potential use of a genetically modified muscle cell line in therapeutic angiogenesis for the treatment of muscle disease associated with vascular defects.

P8.6

MicroRNA induced myogenic differentiation of mouse pluripotent stem cells

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Pluripotent stem cells, such as embryonic stem cells (ESCs), have self-renew capacity and can differentiate into any tissue that builds an organism. Therefore, muscle cells derived from differentiated ESCs hold promise for treating muscle injuries or degenerative diseases. However, so far no efficient protocol for myogenic differentiation of ESCs has been obtained. Our research concerns microRNA as a tool to targeted ESCs differentiation. microRNAs (miRNAs, miRs) are short, single-stranded molecules regulating proliferation and differentiation various types of cells. We aimed to determine the role of miRNAs during in vitro myogenic differentiation of ESCs. Our previous study documented the impact of selected miRNAs at differentiation of D3 and B8 mouse ESCs lines. Currently, we wanted to extend our research to the reporter cell lines what would give us the chance to follow these cells after their transplantation into regenerating muscles. Since pluripotent stem cell lines may vary in their reaction to various treatments, we have to carefully test them. Thus, we chose two mouse ESCs lines, i.e. H2B-EGFP and 7AC5-YFP, and transfected them with miR145 and miR181, then induced myogenic differentiation through selected sequence of changing media and culture methods. Next, we analysed effect of transient miRNA expression on the level of factors characteristic for: paraxial mesoderm (Pdgfrα), myogenic precursor cells (Pax7) or myoblast and myotubes formation (Myf5, Myog). Additionally, we localised the cells expressing skeletal myosin heavy chains (MyHC) specific for muscle tissue. We observed several differences between 7AC5-YFP and H2B-EGFP cells transfected with miR145 or miR181 at the level of the expression of listed markers. In next step we will test other selected miRNAs, which are promising tools to induce ESC myogenic differentiation.

P8.7

Engineered Heart Tissues for testing personalized therapy in hypertrophic cardiomyopathy: the impact on a MYBPC3 mutation with founder effect in Tuscany

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Human engineered heart tissues (EHTs) can be used for *in vitro* disease modelling and potentially developing personalized therapies. As a paradigm, we selected the c.772G>A in *MYBPC3* mutation with founder effect in Tuscany (Italy) causing hypertrophic cardiomyopathy (HCM). In patient myocardium, we found lower MyBP-C levels resulting in accelerated rate of cross-bridge cycling and increased energetic cost of tension. These findings candidate Mavacamten, an allosteric inhibitor of myosin, as potential for reducing the impairment of sarcomere energetics. To this aim, EHTs from a patient hiPSC line were compared with its isogenic CRISPR/Cas9 corrected cell line and a control from a healthy individual. After formation, EHTs are regularly analyzed for spontaneous auxotonic contractions and frequency over a period of 50 days. All EHTs developed greater tension generating capacity and reduced their spontaneous beating frequency at Ca^{2+} [0.4 mM]. At day 50 p.d., EHTs were detached from pillars and used for mechanical investigations in isometric conditions under pacing stimuli and higher calcium concentrations. Under maximal calcium concentration (4 mM), Mavacamten (EC_{50} 1 μM) reduced tension in patient EHTs. In the future, this approach will be applied to evaluate a long-lasting treatment of patient-EHTs with Mavacamten to verify potential prevention of disease mechanisms.

P8.8

The increase in the level of DNA-double strand breaks in iPSCs-derived muscle cells from EDMD1 patients

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Emery-Dreifuss muscular dystrophy type 1 (EDMD1) is a rare genetic disease that belongs to the group of laminopathies. EDMD1 is caused by mutations in the *EMD* gene coding for nuclear envelope protein emerin. It is involved among others in maintaining the structure of the cell nucleus, chromatin organization, DNA damage response (DDR), regulation of gene expression and stress response. The main symptoms of EDMD1 are observed in the skeletal muscles and in the heart, and they are weakness and muscle wasting, tendon contractures, and cardiac dysfunction.

In our studies, we reprogrammed fibroblasts from two EDMD1 patients with mutation c.153delC and healthy donor (control) to induced pluripotent stem cells (iPSCs). This mutation causes frameshift and generation of premature STOP codon. The protein is not detected, so this mutant is considered as emerin-null. Obtained iPSCs from two patients and control were characterized for pluripotency markers and their potential to differentiate into three germ layers were tested. The pluripotency of all clones was confirmed.

iPSCs were used for *in vitro* differentiation into skeletal muscle cells using commercially available media. We investigated the level of DNA double-strand breaks (DSB) in myoblasts and myotubes using antibodies against 53BP1 and γH2AX pS139, which form clusters on sites of double strand breaks. In muscle cells from two patients with mutation c.153delC, we observed the increased level of DNA double-strand breaks in comparison to healthy donor. Our study has shown that mutation c.153delC in *EMD* gene has an influence on higher level of DNA double-strand breaks in EDMD1 patients' muscle cells.

P8.9

Biomimetic matrices and histone deacetylase inhibitors in cardiomyogenic differentiation of dilated myocardium human myocardium-derived mesenchymal stromal cells

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Background. Dilated cardiomyopathy (DCM) is a progressive, often irreversible, disease causing contractile heart muscle dysfunction leading to heart failure. Biomatrices, epigenetic regulators or adjustable geometry cell growth surface can promote cardiac regeneration properties. In this study, the impact of collagen I-based biomatrices, histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) and unidirectional cell growth on cardiomyogenic differentiation of human healthy and dilated myocardium-derived mesenchymal stem/stromal cells (hmMSC) have been investigated.

Methods. hmMSC have been isolated from human healthy and dilated myocardium biopsies and characterized for MSC origin. Cardiomyogenic differentiation of hmMSCs was induced by histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) cultivating cells on: collagen type I hydrogel with hyaluronic acid (HA) or 2-methacryloyloxyethyl phosphorylcholine (MPC) and evaluated at gene and protein levels. The effects of directional cell growth on fibronectin lines without additional stimuli has been also investigated.

Results. hmMSC isolated from healthy and dilated ventricle had MSC-typical surface markers but showed different upregulation pattern of α-cardiac actin *(ACTC1)* and cardiac troponin T *(TNNT2)* genes when cultivated on different composition hydrogels with 1 μM SAHA. Unidirectional cell growth on fibronectin line-patterned surface upregulated α-cardiac actin without additional stimuli.

Conclusion. The data from this study show that cardiomyogenic differentiation of human dilated ventricle-derived hmMSC can be targeted regulated by collagen I-based hydrogel matrices and histone deacetylase inhibitor SAHA.

P8.10

An improved method for culturing myotubes on laminins for the robust clustering of postsynaptic machinery

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Motor neurons form specialized synapses with skeletal muscle fibers, called neuromuscular junctions (NMJs). The laminin-cultured myotubes provide a minimalistic approach for studying the NMJ's postsynaptic machinery, without the context of motor nerves and Schwann cells. An advantage of using laminin-cultured myotubes over other *in vitro* methods is the fact that clusters of the acetylcholine receptors (AChR) in this system undergo developmental remodeling, similar to the one observed *in vivo*. However, the molecular mechanisms that drive the assembly of the receptors into specialized structures remain largely unknown for model organisms such as mouse, and completely undiscovered for humans. Here, we describe an improved protocol for culturing muscle cells to promote the formation of the complex AChR clusters. We screened eight laminin isoforms and showed that laminin-221 (α2, β2, γ1) was the most potent for inducing AChR cluster formation, whereas laminin-121 afforded the highest percentages of topologically complex assemblies. We also applied our method to primary human myoblast cultures and found that these cells can efficiently form AChR clusters on any of the tested laminin isoforms. Our new protocol may facilitate findings of new molecular regulators of the synapse, while primary human muscle cells may become a more reliable tool for screening new therapeutic agents and studies that aim to diagnose neuromuscular disorders.

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P8.11

A hypertrophic cardiomyopathy myosin binding protein C mutation with founder effect causes impairment of crossbridge kinetics and energetics: a clinical and in vitro study

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Mutations in *MYBPC3* are the most common cause of hypertrophic cardiomyopathy (HCM). The MYBPC3-c772G>A is a highly prevalent mutation in Tuscany (Italy). However, its functional impact on human has never been explored. To this aim, we used myocardial samples and induced pluripotent stem cell (hiPSC)-derived cardiomyocytes from the same patients. Here we report by haplotype analysis that the highly prevalent (*MYBPC3*)-c772G>A is indeed a founder mutation in Tuscany. We collected myocardial samples from three subjects of our cohort with preserved ejection fraction who underwent myectomy before the onset of end-stage cardiomyopathy. We compared a hiPSC line from one of this patients with its isogenic CRISPR-Cas9 corrected control. Patient trabeculae and engineered heart tissues were analyzed. The mutation leads to a reduction of cMyBP-C expression, likely supporting the hypothesis of haploinsufficiency as the main underlying mechanism. The subsequent effects of the mutation include i) faster cross-bridge cycling and higher energy cost of tension generation ii) prolonged action potential and Ca^{2+} transient duration iii) preserved twitch duration. Patient myocardium and hiPSC-cardiomyocytes allowed us to demonstrate that the HCM-related MYBPC3-c772G>A mutation invariably impairs sarcomere energetics and cross bridge cycling, but concurrent E-C coupling changes can preserve twitch contraction parameters, setting the stage for genotype-driven preventive therapeutical interventions.

P8.12

The role of ceramide in the reorganization of the sarcolemma and oxidative stress during functional unloading of skeletal muscles

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The inactivity of skeletal muscles can be accompanied by an increase in the level of ceramide and oxidative stress in muscle fibers (Bryndina I. G. *et al.*, 2020; Powers S. K. *et al.*, 2007). We have suggested that these processes may be associated with changes in lipid rafts in the sarcolemma. The aim of this work was to investigate the effect of the inhibitor of aSMase clomipramine on the amount and localization of NADPH-oxidase 2 (NOX2), NADPH-oxidase 4 (NOX4) and marker of lipid rafts Flotillin-1 (Flot-1) in rat soleus muscle at 14 days of HS. All experiments were performed on male albino rats, which were divided into three groups: control rats, hindlimb suspeneded group (HS) and rats subjected HS and treated with clomipramine. We used immunofluorescence and WB methods to study changes NOX2, NOX4 and Flot-1 on rat soleus muscle. We found the increase of NOX2, NOX4 and Flot-1 amount in unloaded during 14 days m. soleus. Treatment with clomipramine prevented both NOX2, NOX4 and Flot-1 accumulation in unloaded muscle. These changes may indicate that the development of oxidative stress and lipid raft reorganisation in disused skeletal muscle may be associated with aSMase/Cer mechanisms.

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P8.13

microRNA-378 regulates metabolism of hiPSC-derived cardiomyocytes by modulation of mitochondrial content

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MicroRNA-378a (miR-378a), encoded in the first intron of *PPARGC1B* gene, is highly expressed in tissues with high metabolic demands including brown fat, skeletal muscles and heart. Within the latter organ, it is predominantly found in cardiomyocytes and several studies already indicated its cardioprotective and metabolism-regulating activity in rodents. The effect of miR-378a on the metabolic properties of human cardiomyocytes, however, has not been described.

For that purpose, we have first generated miR-378a-deficient human induced pluripotent stem cell lines (miR-378aKO hiPSC) using CRISPR/Cas9 gene editing method. Particularly, application of Cas9 and two sgRNA sequences targeting flanking regions of miR-378a locus provided complete removal of this microRNA. In the next step, control and miR-378aKO hiPSC were subjected to cardiac differentiation triggered by small molecules regulating WNT pathway. Seahorse Cell Metabolic Analysis performed on obtained cells (hiPSC-CM) revealed decreased value of Oxygen Consumption Rate (OCR) in miR-378a-deficient hiPSC-CM (miR-378aKO hiPSC-CM) in comparison to isogenic control counterparts. Moreover, we have observed lower mitochondrial membrane activity and ratio of mitochondrial to genomic DNA (mtDNA/gDNA) in these cells. Electron microscope imaging showed additionally that mitochondria in miR-378aKO hiPSC-CM are larger and swelled in comparison to control cells. Accordingly, upon miR-378a silencing in control hiPSC-CM, we have observed that levels of TFAM and NRF-1, two factors involved in mitochondrial biogenesis, are decreased.

To conclude, our results indicate that miR-378a regulates cardiac metabolism by regulation of mitochondrial biosynthesis and function.

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P8.14

Dietary Intervention delays the onset of Sarcopenia in *Caenorhabditis elegans*

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Sarcopenia, a progressive decrease of skeletal muscle mass and strength, is one of the primary changes associated with ageing. Multiple factors are responsible for development of sarcopenia, such as hormonal changes, loss of neurons, physical inactivity, and an unbalanced diet. This study aims to determine the effect (if any) of dietary intervention on the rate of muscle loss, using *C. elegans* as a model system. The specific strain used (*unc54:GFP*) has its major myosin isoform (UNC-54) in body wall muscle fluorescently labelled, allowing UNC-54 myosin levels to be measured throughout *C. elegans'* lifespan.

Fluorescence intensity increases during the first eight days of adulthood, representing muscle development, then decreases for all dietary groups. From day 10, significant differences in fluorescence intensity were found for mild and medium dietary restriction (DR) groups compared to control ($p<0.05$, 2-way ANOVA). Mild and medium DR groups lost 50% of their maximum fluorescence at 15.3 days and 14.8 days respectively, compared to control and severe DR groups which lost 50% of peak fluorescence at 12 days and 13.1 days, respectively.

The decrease in fluorescence, associated with reduced myosin UNC54 levels, is confirmed by motility data. Non-linear regression analysis indicated that the amount of time spent in motility class I,II and III differed across groups, with mild and medium DR worms spending more days in motility classes I ($C_{1\text{-}2}$ = 8.6 and 8.5 days) and II ($C_{2\text{-}3}$= 15.4 and 14.3 days) than severe DR and control ($C_{1\text{-}2}$ = 6.9 days,$C_{2\text{-}3}$= 12.0 and 10.0 days, respectively). Collectively, these results show a delayed onset of sarcopenia under mild and medium DR, compared to control and severe DR.

P8.15

Impact of gravitational unloading on muscle satellite cells

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Skeletal muscle has a unique ability to regenerate and repair the damaged fibers. Muscle regeneration potential is believed to be associated with the presence of muscle satellite cells. Regeneration of postural muscles under conditions of gravitational unloading is poorly investigated. Previously it was shown that gravitational unloading leads to decline of the total number of satellite cells and a decrease of their proliferation rate. The aim of the study was to investigate the regenerative potential of muscle satellite cells at the different stages of gravitational unloading. Wistar rats were subjected to 1-day, 3-day, 7-day and 14-day hindlimb suspension (HS). Further was assessed the expression of proliferation and differentiation markers of muscle satellite cells and MHC expression by RT-PCR analysis. Muscle satellite cells proliferation rate was measured by EDU incorporation.

After 7 and 14-days of HS we observed an increase in Myf 5, MRF4, and MyoG mRNA expression *vs.* control group. While an expression level of the markers didn't differ from control after 1 and 3 day of HS. 7 and 14-day of HS resulted in a significant increase in eMyHC, MHCIIb and MHCIIdx mRNA expression. Also we found a decline in satellite cells proliferation rate after 1, 3 and 7 days of HS, after 14 days of HS we observed an increase of satellite cells proliferation.

Thus, activation of muscle satellite cells at the stage of their differentiation was increased after the relatively long exposures to simulated gravitational unloading.

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