Session 9: Muscle and beyond

Lectures

L9.1

Acetylcholine receptor turnover at the neuromuscular junction: Role of cAMP-microdomain and sympathetic regulation

R. Rudolf[1,2]

[1]Institute of Molecular and Cell Biology, Mannheim University of Applied Science, Mannheim, Germany; [2]Interdisciplinary Center for Neuroscience, Heidelberg University, Heidelberg, Germany
Rüdiger Rudolf <r.rudolf@hs-mannheim.de>

As synapses between motoneurons and skeletal muscle fibers, neuromuscular junctions (NMJ) mediate skeletal muscle contraction and are therefore of utmost physiological importance. In this context, nicotinic acetylcholine receptors (nAChR) serve as major ligand-gated ion channels to convert motoneuron firing into postsynaptic action potential and, thus, to trigger excitation-contraction coupling. It has been known that turnover of nAChR is subject to massive activity-dependent regulation. This involves shifts of the kinetics of receptor clustering at the postsynaptic membrane, endocytic retrieval, activity-dependent recycling, and degradation. Live muscle imaging combined with the use of molecular fluorescent biosensors shed light on the underlying mechanisms of nAChR protein trafficking, such as (i) the role of a specific cAMP microdomain at the NMJ, (ii) the specific function of rapsyn as a scaffold protein in the microdomain, (iii) the cooperation between recycling vesicles and the motor protein, myo5a, and (iv) the involvement of the membrane shaping protein, endophilin B1, and the E3-ligase, MuRF1, in endocytosis and selective autophagy of nAChR on the route to degradation. Finally, recent studies showed that these processes are modulated by sympathetic neurons. These were found to critically participate by direct innervation in the neuromuscular crosstalk and the maintenance and function of NMJs.

L9.2

Skeletal muscle phenotype and function conducted by CNS orchestra

U. Sławińska

Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland
Urszula Sławińska <u.slawinska@nencki.edu.pl>

In tribute to Gerta Vrbová, who made a unique contribution in introducing the great concept of activity-related neuromuscular plasticity prompting changes of the skeletal muscle phenotype and contractile properties.
We investigated the hindlimb muscle plasticity in the model of rat paraplegia. Total spinal cord transection at the thoracic level in adult rats induces severe impairment of locomotor hindlimb functions. Our results demonstrate that such considerably reduced muscle activity affects the slow postural soleus muscle to a greater extent than the fast phasic tibialis anterior (TA) muscle. On the other hand, the postural medial gastrocnemius (GM) muscle similarly to the TA phasic muscle becomes slower, weaker, and more fatigable after total spinal cord transection. However, the GM turns out to be composed of a higher proportion of fast IIx and IIb MHC isoforms, while TA of a higher proportion of type IIx muscle fibers while type IIb is significantly reduced. Thus, although the GM and TA muscles are both fast, the postural extensor GM muscle is more affected by reduced neuromuscular activity than the phasic flexor TA one.
In paraplegic rats, the recovery of impaired locomotor hindlimb function can be enhanced by re-establishing monoaminergic innervation of the spinal cord below the total transection using intraspinal grafting of embryonic raphe nuclei (E14). Our investigations show, however, that improved neuromuscular activity influenced the typical characteristics of the contractile properties of slow postural muscle (Soleus) to a greater extent than that of the fast phasic (TA) muscle.
Thus, activity-dependent muscle transformation of rat muscles is determined to some extent by the muscle functional demands (postural *vs.* phasic muscle).

L9.3

Disrupted T-tubular network accounts for asynchronous Ca^{2+} release in MTM1 deficient skeletal muscle

L. Csernoch[1], P. Szentesi[1], C. Kutchukian[2], A. Buj-Bello[3], B. Dienes[1], V. Jacquemond[2]

[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [2]University of Lyon, Université Claude Bernard Lyon 1, CNRS UMR-5310, INSERM U-1217, Institut NeuroMyoGène, Lyon, France; [3]INSERM U951, Evry, University of Evry, UMR_S951, France

Laszlo Csernoch <csl@edu.unideb.hu>

In skeletal muscle propagation of membrane depolarization (MD) into the muscle fibre *via* the transverse tubules (TT) is essential for synchronized release of Ca^{2+} from the sarcoplasmic reticulum (SR). Ca^{2+} release occurs via Ryanodine receptors (RyR) in response to the conformational change in the voltage-sensing dihydropyridine receptors (DHPR). The conformational change in DHPR gives rise to an intramembrane charge displacement (ICD). Deficiency in the 3-phosphoinositide phosphatase myotubularin (MTM1) was reported to result in the disruption of TT and the appearance of delayed SR Ca2+ release. Here Ca^{2+} transients were recorded in skeletal muscle fibres of MTM1 deficient mice isolated from the *m. flexor digitorum brevis* using Rhod-2 and a confocal microscope. Propagation of the depolarization along the TT was modelled mathematically. Disruptions in TT were assumed to modify their resistance and capacitance. The estimated MD was then used to calculate ICD and the consequent release of Ca^{2+}. The latter was compared to measured Ca^{2+} transients. If, in simulations, TT were assumed to be partially or completely inaccessible for the depolarization and RyR at these points to be prime for Ca^{2+}-induced Ca^{2+} release all disease features of measured SR Ca^{2+} release detected in MTM1-deficient fibres could be reproduced. We conclude that the inappropriate propagation of the depolarization into the fibre interior is the cause of severely impaired SR Ca^{2+} release in MTM1 deficiency.

Oral Presentations

O9.1

AMBRA1 deficiency impairs mitophagy in skeletal muscle

L. Gambarotto[1,2], S. Metti[1], M. Chrisam[1], C. Cerqua[3], P. Sabatelli[4], A. Armani[5], S. Castagnaro[1], P. Grumati[1], F. Strapazzon[6], P. Braghetta[1], F. Cecconi[7], P. Bonaldo[1]

[1]Department of Molecular Medicine, University of Padova, Padova, Italy; [2]Department of Biology, University of Padova, Padova, Italy; [3]Pediatric Research Institute-IRP Città della Speranza, Padova, Italy; [4]IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy; [5]Department of Biomedical Sciences, VIMM, University of Padova, Padova, Italy; [6]IRCCS Fondazione Santa Lucia, Rome, Italy; [7]Danish Cancer Society Research Center, Copenhagen, Denmark

Lisa Gambarotto <lisa.gambarotto@phd.unipd.it>

The proper function of autophagy is critical for the maintenance of muscle homeostasis. AMBRA1, whose deficiency is embryonic lethal and leads to severe impairment in neuromuscular development, is a well-known autophagic and mitophagic regulator. Within this context, we aimed at dissecting the roles of AMBRA1 in adult skeletal muscle, focusing on the mitochondrial compartment and its selective degradation.

We found that the *in vivo* overexpression of a mitochondria-targeted form of AMBRA1 in muscles results in enhanced mitophagic flux. Thus, we generated muscle-specific *Ambra1* knockout mice, and found that they display a significant decrease of myofiber cross sectional area and of oxidative fibers. AMBRA1-depleted myofibers show impaired regulation of the endo-lysosomal compartment, as well as abnormal accumulation of mitochondria, both confirmed by ultrastructural analyses. Functional studies on AMBRA1-depleted myofibers revealed mitochondrial dysfunction, with reduced oligomycin-dependent hyperpolarization and significantly lower complex I-III activity. Lack of AMBRA1 leads to impaired mitophagic flux, with lower amounts of DRP1 and Parkin recruited to mitochondria.

These findings unravel new *in vivo* roles of AMBRA1 in adult tissues, strongly supporting the novel concept that AMBRA1 is involved in the turnover of mitochondria in muscle, leading to the accumulation of disrupted mitochondria when it is missing.

O9.2

The positive modulation of the Mitochondrial Calcium Uniporter activity by Amorolfine sustains skeletal muscle trophism

Agnese De Mario[1], Anna Tosatto[1], Gyorgy Szabadkai[1,2], Rosario Rizzuto[1], Cristina Mammucari[1]

[1]Department of Biomedical Sciences, University of Padua, Padua, Italy; [2]Department of Cell and Developmental Biology, Consortium for Mitochondrial Research, University College London, London, UK

Agnese De Mario <agnese.demario@unipd.it>

Mitochondria represent fundamental checkpoints for muscle homeostasis, since they are a major source of ATP production, thus sustaining muscle activity and functions. Skeletal muscle mitochondria readily accumulate Ca^{2+} in response to SR store-releasing stimuli thanks to the activity of the mitochondrial calcium uniporter (MCU), the highly selective channel responsible for mitochondrial Ca^{2+} ($mitCa^{2+}$) uptake. In skeletal muscle, MCU-dependent $mitCa^{2+}$ positively regulates myofiber size by impinging on PGC1α4 and IGF1-AKT/PKB pathways. While the genetic modulation of the MCU has been widely applied, small molecules able to increase $mitCa^{2+}$ uptake are rare. By using a well-established methodology based on Aequorin, a calcium-sensitive probe that emits light upon Ca^{2+} binding, we screened a library of 1,600 FDA-approved drugs for their ability to modulate $mitCa^{2+}$ uptake in living cells. We identified Amorolfine as a positive MCU modulator. Amorolfine is a morpholine antifungal drug that inhibits enzymes of the fungal sterol synthesis pathway and it is indicated for the topic treatment of mycoses. Amorolfine increases $mitCa^{2+}$ uptake in Hela, C2C12 cells and adult isolated myofibers without affecting $cytCa^{2+}$ and mitochondrial membrane potential. In agreement with the role of MCU in triggering hypertrophy, Amorolfine increases the size of C2C12 myotubes in an MCU-dependent manner, and triggers muscle hypertrophy *in vivo*. Thus, these data indicate that Amorolfine, by modulating MCU, positively regulates muscle trophism. In the future, we aim to verify whether Amorolfine treatment could exert a protective effect against skeletal muscle atrophy and sarcopenia.

O9.3

MicroRNA-378 loss mediates systemic metabolic changes in the *mdx* model of Duchenne muscular dystrophy

P. Podkalicka[1], O. Mucha[1], K. Szade[1], J. Stępniewski[1], L. Ivanishchuk[1], K. Kaziród[1], E. Pośpiech[2], J. Dulak[1#], A. Łoboda[1#]

[1]Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland; [2]Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland

Paulina Maria Podkalicka <paulina.podkalicka@doctoral.uj.edu.pl>

[#]Equally contributed as senior authors

Although Duchenne muscular dystrophy (DMD) affects primarily muscle tissues, alterations in systemic metabolism manifested by DMD patients contribute to the severe phenotype of this fatal disorder. We propose that microRNA-378 (miR-378) with a documented role in metabolism mediates metabolic dysfunctions exhibited by dystrophic *mdx* mice.
In our study, we utilized double knockout animals lacking both dystrophin and miR-378 (*mdx*/miR-$378^{-/-}$) and focused mostly on poorly described hepatic dysregulation of carbohydrate and lipid homeostasis regulators. RNA sequencing performed for the first time in the liver identified 561 and 194 differentially expressed genes that discriminated *mdx* against WT and *mdx*/miR-$378^{-/-}$ *versus mdx* livers, respectively. Bioinformatic analysis predicted metabolic disorders in dystrophic mice what we functionally proved by impaired glucose tolerance and insulin sensitivity. The lack of miR-378 in *mdx* mice mitigated those effects by a faster glucose clearance. This was accompanied by altered glycogen content and lipid homeostasis mediators in livers of *mdx* and *mdx*/miR-$378^{-/-}$ animals.
In conclusion, we report for the first time that miR-378 mitigates metabolic dysfunctions exhibited by *mdx* mice. Together with our previous finding demonstrating alleviation of muscle-related symptoms of DMD (Podkalicka *et al.*, 2020, *JCI Insight*), we propose that inhibition of miR-378 may represent a new strategy to attenuate multifaceted symptoms of DMD.

Acknowledgements of Financial Support: Supported by PRELUDIUM 16 (2018/31/N/NZ4/02500 to PP), MAESTRO 3 (2012/06/A/NZ1/00004 to JD), and MAESTRO 10 (2018/30/A/NZ3/00412 to JD) grants. PP is a recipient of the START scholarship for outstanding young scientists funded by the Foundation for Polish Science.

Virtual Posters

P9.1

Involvement of myosin VI is in the murine neuromuscular junction structural remodeling

Paloma Alvarez-Suarez, Maria J. Rędowicz

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
Paloma Alvarez-Suarez <alvarez925@gmail.com>

Unconventional myosin VI (MVI) is a unique motor protein that moves backwards along actin filaments. MVI's singularity accounts for its multiple roles in non-muscle cells, such as clathrin-mediated endocytosis, intracellular vesicle transport, and actin cytoskeleton dynamics. More recently, MVI has been associated with myoblast adhesion, fusion and differentiation. In mice, spontaneous mutation of a single gene (*MYO6*) encoding MVI leads to a non-functional protein, which results in impaired cardiac tissue growth and BDNF-mediated neurotransmission, to name a few. However, little is known about the role of MVI at the neuromuscular junction (NMJ), where it has been previously localized to the postsynaptic compartment. In this study, we characterized the NMJs of MVI knockout mice throughout their lifespan by analyzing whole end-plate morphology as well as postsynaptic fragmentation and disruption. We observed that the MVI knockout animals have neuromuscular defects at developmental stages in which NMJs undergo structural remodeling (i.e. during early postnatal maturation and ageing). Our preliminary results suggest that MVI might play a role in the postsynaptic machinery organization, but further research is needed to understand the molecular mechanisms underlying the function of this motor protein.

Acknowledgements of Financial Support: This research was supported by the Polish National Science Centre grants UMO-2017/27/B/NZ3/01984 and UMO-2018/29/N/NZ3/02682.

P9.2

CRISPR-Cas9 correction of out-of-frame exon 2 duplication in iPSCs from patients with Duchenne Muscular Dystrophy

I. N. Barbosa[1], F. de Souza Leite[1,2,3], J. Esposito[1], M. C. S. Pardo[1], T. M. M. Martins[1], G. G. O. Olberg[1], M. C. L. Pereira[1], R. H. Bortolin[4], M. Zatz[1]

[1]Human Genome and Stem Cell Research Center, University of São Paulo, São Paulo, Brazil; [2]Boston Children's Hospital, Harvard Medical School, Boston, USA; [3]Brigham & Women's Hospital, Harvard Medical School, Boston, USA; [4]Department of Clinical and Toxicological Analysis, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil
Igor Neves Barbosa <nvs.igor@usp.br>

Introduction: Duchenne Muscular Dystrophy (DMD) is caused by disruption of dystrophin expression due to frame-shifting or truncating mutations in the *DMD* gene. Among the disease-causing mutations, approximately 31.5% could be corrected via CRISPR/Cas9 gene editing. Here, we rescued dystrophin expression in iPSC-derived skeletal muscles from two siblings carrying the same duplication in the *DMD* exon 2. **Methods**: We reprogrammed fibroblasts obtained from patients' skin biopsies into induced Pluripotent Stem Cells (iPSCs). Next, patients' iPSCs were transfected with Cas9 protein and a gRNA (ribonucleoprotein complex) targeting the duplicated subjacent intron to correct the duplication. The gene correction was assessed through PCR at the duplication breakpoint and Multiplex Ligation-dependent Probe Amplification (MLPA). Then, control and edited iPSC clones were differentiated into skeletal muscles using a transgene-free method. We evaluated dystrophin expression in the differentiated muscle by Western Blotting and Immunolabeling. **Results**: Dystrophin expression was recovered in skeletal muscle cells differentiated from *DMD*-corrected iPSCs. **Conclusion**: We provide a glimpse into the possibilities of gene correcting iPSCs generated from patients in order to develop personalized cell therapies for DMD and other diseases.

P9.3

Effects of ageing and exercise on the structure of human skeletal muscle nuclei

Ed Battey

King's College London, UK
Ed Battey <edmund.battey@kcl.ac.uk>

Age-related declines in cellular structure and function can be initiated or accelerated by physical inactivity. Ageing is associated with aberrant nuclear morphology, architecture and translation of forces to biochemical signals (mechanotransduction); this project focuses on whether, in skeletal muscle, these changes are influenced by endurance training status. Nuclear morphology and the distribution of nuclear envelope proteins important for nuclear architecture and mechanotransduction (SUN1 and Lamin A) were studied in isolated human muscle fibres from individuals of different ages and endurance training statuses. So far, the data indicate that in humans, changes in myonuclear morphology occur in response to exercise regardless of age, indicated by a 6-10% reduction in myonuclear sphericity and 25-30% reduction in myonuclear aspect ratio in young and older exercise-trained individuals compared to inactive young and older counterparts. No differences in the distribution of SUN1 or Lamin A were revealed through super-resolution and confocal microscopy. Endurance training therefore appears to influence nuclear morphology in a manner unaffected by age; alterations to nuclear envelope proteins other than SUN1 may be involved in this process. Ongoing research is focused on whether these changes are recapitulated in mice and if the distribution of nuclear envelope protein Nesprin-1 and muscle-specific cytoskeletal protein Desmin are altered with endurance training. Whether alterations to myonuclear mechanics influences gene transcription will also be assessed through cell microharpooning and RNA sequencing.

P9.4

miRNA-10a, miRNA-425, and miRNA-5100 impact myogenic stem and progenitor cells differentiation

B. Mierzejewski, I. Grabowska, K. Zduńczyk, Z. Michalska, F. Zaremba, M. Chrząszcz, M. Patrycy, W. Stremińska, K. Jańczyk-Ilach, M. A. Ciemerych, E. Brzoska

Department of Cytology, Faculty of Biology, University of Warsaw, Warsaw, Poland
Edyta Brzoska-Wojtowicz <edbrzoska@biol.uw.edu.pl>

Reconstruction of skeletal muscle fibers requires myogenic stem cells, i.e., satellite cells (SCs), activation and differentiation. However, other cell types, such as fibroblasts, endothelial cells, vascular smooth muscle cells or resident and infiltrating inflammatory cells, also participate in the restoration of skeletal muscle structure. Recently, we focused on the progenitor cells that reside within skeletal muscle interstitium, between myofibers but outside basal lamina or in the vicinity of blood vessels. These cells, referred to as muscle interstitial progenitor cells (MIPCs), express inter alia selected pericyte markers. Based on our previous experiments, miRNA-10a, miRNA-425, and miRNA-5100 were selected as potentially significant in cell migration. Thus, SCs and MIPCs were transfected with miRNA mimics, i.e., chemically modified double-stranded RNA molecules that mimic endogenous miRNAs (miRNAs), encoding these molecules. Cell migration, differentiation *in vitro* and *in vivo*, and transcriptome changes were analyzed. We showed that the abovementioned miRNAs impacted SC and MIPC myogenic differentiation in NOTCH dependent manner.

P9.5

Pharmacological modulation of purine and folate metabolism as a gateway to activation of AMP-activated protein kinase in cultured L6 myotubes

K. Dolinar[1], K. Miš[1], K. Šopar[1], M. Kolar[1], T. Hropot[1], M. Šutar[1], P. M. Garcia-Roves[2], A. V. Chibalin[3], S. Pirkmajer[1]

[1]Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [2]Department of Physiological Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain; [3]Department of Molecular Medicine and Surgery, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden
Klemen Dolinar <klemen.dolinar@mf.uni-lj.si>

Introduction: Insulin resistance in skeletal muscle, a major site of glucose disposal, underlies hyperglycaemia in type 2 diabetes. Activation of AMP-activated protein kinase (AMPK), which stimulates glucose uptake and enhances insulin action, is a promising strategy for the treatment of diabetes. We previously showed that antirheumatic drug methotrexate (MTX) promotes AMPK activation and glucose uptake in cultured myotubes. By inhibiting folate and purine metabolism, MTX suppresses conversion of ZMP to IMP, leading to accumulation of ZMP, which directly activates AMPK.
Aim: To investigate whether mycophenolate mofetil (MMF) and mercaptopurine (MP), which inhibit IMP metabolism, and trimethoprim (TMP) and trimetrexate (TMX), which inhibit folate metabolism, mimic effects of MTX on AMPK and glucose uptake in L6 myotubes. We also investigated effects of these drugs on insulin actions.
Results: MMF reduced basal glucose uptake without altering AMPK activity or insulin signalling. MP increased AMPK activity and glucose uptake and suppressed insulin signalling. TMP and TMX had almost no effect on AMPK activity, insulin signalling and glucose uptake. MMF and TMX enhanced stimulation of AMPK activity and glucose uptake by pharmacological ZMP precursor AICAR.
Conclusions: MMF, MP and TMX increased basal or AICAR-stimulated AMPK activity and glucose uptake, suggesting that modulation of purine and/or folate metabolism may serve as a gateway to activation of AMPK in skeletal muscle.

P9.6

Dynamics of skeletal muscle lipid droplets during the development of grass snake (*Natrix natrix* L.)

Magda Dubińska-Magiera[1], Damian Lewandowski[1], Dominik Cysewski[2], Seweryn Pawlak[1], Bartłomiej Najbar[3], Małgorzata Daczewska[1]

[1]Department of Animal Developmental Biology, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland; [2]Mass Spectrometry Laboratory, IBB PAS, Warsaw, Poland; [3]Faculty of Biological Sciences, University of Zielona Góra, Szafrana 1, 65-516, Zielona Góra 1, Poland
Magda Dubińska-Magiera <magda.dubinska-magiera@uwr.edu.pl>

Lipid droplets (LDs) are multifunctional organelles. Their main function is the storage and turnover of neutral lipids. LDs consist of a neutral lipid core surrounded by a phospholipid monolayer with proteins embedded in or bound to it which are necessary to droplet structure and function. The presence of LDs in developing muscle cells was observed by us previously during grass snake myotomal myogenesis [1].
Here we report the results of our detailed morphometric analysis which provided information regarding the dynamics of *N. natrix* skeletal muscle LDs. Additionally, we performed quantitative lipidomics analyses of LDs isolated from the muscles of the snake's embryos at subsequent developmental stages. Through IF and LC-MS analyses, we identified a variety of proteins associated with snake skeletal muscle LDs. We also conducted skeletal muscle LD-focused ultrastructural analysis.
Our study describes for the first time the dynamics of skeletal muscle LDs during the development of the grass snake and provides their detailed characteristics. Observed dynamic changes are manifested by alternations in the number, size, and composition of LDs.

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Lewandowski M, Dubińska-Magiera E, Posyniak W, Rupik M, Daczewska (2017) Does the grass snake (*Natrix natrix*) (Squamata: Serpentes: Natricinae) fit the amniotes-specific model of myogenesis? *Protoplasma* **254**: 1507-1516.

Acknowledgements of Financial Support: This work was supported by the National Science Centre, Poland (2017/24/C/NZ3/00117).

P9.7

Effect of spinal cord stimulation on the development of atrophic processes in rat leg muscles during hind limb unloading

A. Fedianin, T. Zaytceva, M. Baltin, T. Baltina, A. M. Eremeev, A. A. Eremeev

Department of animal and human physiology, Kazan Federal University, Kazan, Russia
Artur Fedianin <artishock23@gmail.com>

Restriction of muscle functional activity as a result of musculoskeletal damage, CNS injuries, in conditions of weightlessness, is invariably accompanied by the development of atrophy, the prevention of which is an urgent task of neurophysiology and medicine. In the present study, we evaluated the effect of activation of spinal cord neuronal networks in rats on the development of hind limb muscle atrophy while limiting their functional activity. Laboratory animals were divided into the following experimental groups: UN - functional muscle unloading; UN+EES – muscle unloading and daily epidural spinal cord stimulation; UN+MS - muscle unloading and daily non-invasive magnetic spinal cord stimulation. Functional muscle unloading was modeled by hanging the hind limbs. Spinal cord stimulation (EES and MS) was carried out at the L2-L3 level for 90 minutes (10 min stimulation, 10 min break), with a frequency of 3 Hz, threshold intensity for hind limb muscle contraction (determined individually). After 7, 14 and 35 days of exposure to experimental conditions, the wet and dry weights of the soleus, gastrocnemius, and tibialis anterior muscles were determined. It was found that the most pronounced atrophy, manifesting after 7 days of unloading and increasing by 35 days, is observed in the tonic soleus muscle. Weight reduction of mixed gastrocnemius and fast tibialis anterior muscles is observed after 14 and 35 days of unloading. Electrical and magnetic stimulation of the spinal cord limits, but does not prevent, the development of atrophic processes in the hind limb muscles.

Acknowledgements of Financial Support: The reported study was funded by RFBR according to the research project No. 19-04-01067.

P9.8

Hydrogen sulfide as the potential therapy for Duchenne muscular dystrophy

K. Kaziród, P. Podkalicka, O. Mucha, J. Dulak, A. Łoboda

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
Katarzyna Kaziród <katarzyna.kazirod@doctoral.uj.edu.pl>

Duchenne muscular dystrophy (DMD) is an inherited X-linked neuromuscular disorder caused by a lack of functional dystrophin, as the result of more than 7000 patient-specific mutations in the largest human genes, *DMD*. We hypothesized that hydrogen sulfide (H_2S), through its antioxidant, proangiogenic, and anti-inflammatory properties, may potentially provide a novel therapeutic strategy to attenuate DMD pathology.
Proteomic analysis revealed that the level of cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (MPST), the enzymes generating H_2S, is lower in the diaphragm of 6-week-old *mdx* mice, a model of DMD. RNA-seq analysis indicated that endogenous H_2S biosynthesis is diminished also in muscle satellite cells isolated from dystrophic animals. Daily treatment with 100 μmol/kg body weight of NaHS (rapid H_2S donor) for 4 weeks neither affected body weight nor influenced complete blood cell count in *mdx* animals. Although we did not observe any improvement in the grip strength, the activity of creatine kinase, a serum marker of muscle injury, tended to be decreased after NaHS delivery. The protein level of osteopontin, a recently described biomarker of DMD associated with regeneration, inflammation, and fibrosis, elevated in *mdx* mice, was decreaesd by NaHS treatment. Morever, H_2S donor restored decreased expression of proangiogenic factors in *mdx* animals. Ongoing experiments are focused on investigating whether this gaseous mediator can mitigate muscle-related symptoms of DMD.

Acknowledgements of Financial Support:This work was supported by grant No. 2019/35/B/NZ3/02817 (AŁ) from National Science Centre.

P9.9

Apolipoprotein A-I during the grass snake (*Natrix natrix*) muscle development

Damian Lewandowski[1], Magda Dubińska-Magiera[1], Seweryn Pawlak[1], Dominik Cysewski[2], Barłomiej Najbar[3], Małgorzata Daczewska[1]

[1]Department of Animal Developmental Biology, Faculty of Biological Sciences, University of Wroclaw, Sienkiewicza 21 Str., 50-335 Wroclaw, Poland; [2]Mass Spectometry Laboratory, IBB, PAS, Pawinskiego 5a Str., 02-106 Warsaw, Poland; [3]Department of Botany and Ecology, University of Zielona Gora, Szafrana 1 Str., 65-516 Zielona Gora, Poland
Damian Lewandowski <damian.lewandowski@uwr.edu.pl>

The lipid droplets (LDs) are universal cytoplasmic structures observed in cells of organisms. It is accepted that LDs are involved in storage and turnover of neutral lipids. In mammals, LDs are the most prominent in adipose tissue, however, these organelles are observed in other cell types including skeletal muscles. Previous proteomic studies of skeletal muscle revealed over 300 lipid droplet – associated proteins, including proteins involved in LDs metabolism and, surprisingly, the major apolipoprotein of high-density lipoprotein (HDL) – apolipoprotein A-I (apo A-I) [1]. Our previous studies of the grass snake showed the presence of a unique class of muscle fibers where numerous LDs were accumulated [2].

The LDs function in skeletal muscle was not studied in details so far, despite of its undoubtful importance. To investigate the potential function of apolipoprotein A-I in the grass snake muscle fibers we performed the immunocytochemical, Western blot, and LC/MS analysis. To study the changes of cholesteryl esters level, we did the quantitative analysis of lipid droplets isolated from differentiating trunk muscles at subsequent developmental stages.

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Acknowledgements of Financial Support: We acknowledge the support of the National Science Centre, Poland (Project No. 2017/24/C/NZ3/00117).

P9.10

MicroRNA-100/101 reduce bovine primary cell myogenesis andaugment intramuscular lipid deposition by modulating IGF-1R/PROX1

B. A. Mir[1,2], E. Albrecht[1] , O. Hansson[2], S. Maak[1]

[1]Institute of Muscle Biology & Growth, Leibniz Institute for Farm Animal Biology (FBN), W.-Stahl-Allee 2 D-18196 Dummerstorf, Germany; [2]Department of Clinical Sciences, Diabetes & Endocrinology, Lund University, University Hospital Malmö, SE-20502, Malmö, Sweden
Bilal Ahmad Mir <bilal.mir@med.lu.se>

Previously, microRNA-100/-101 and their putative mRNA targets Insulin-like growth factorreceptor-1 (IGF1R) and prospero-related homeobox 1 (PROX1) respectively, were identifiedas differentially expressed in bovine musculus longissimus dorsi with varying intramuscularfat content by our group. The IGF1R signalling and PROX1 are implicated in myogenesis andlipid metabolism in muscle. However, the underlying regulatory mechanisms are poorlyunderstood. MicroRNAs (miRNAs) regulate, posttranscriptionally, vital biological processesincluding muscle development and metabolism by binding to target mRNAs. In the presentstudy, we aimed to investigate regulation of above mentioned target genes by predictedmiRNAs during bovine primary muscle cell proliferation and differentiation. MiR-100/-101were confirmed to target IGF1R and PROX1 seed sequences by luciferase reporter assay. Furthermore, expression of miR-100/-101 and, IGF1R and PROX1 was reciprocal duringbovine primary muscle cell differentiation, suggesting a simultaneous cross-talk between miRNAs and target genes. Correspondingly, mimic and inhibitor induced miRNA expressionin primary myotubes suppressed or stimulated transcript and protein levels of target andmyogenic genes including MYOG and MYOD. Oleic acid induced lipid deposition in primarymuscle myotubes was increased by miRNA overexpression and decreased by inhibition. Moreover, mitochondrial beta-oxidation and long chain fatty acid synthesis related geneswere modulated by this intervention. Whether IGF1R reciprocally regulates miR-100 wasdetermined by the IGF1R inhibitor (BMS-754807) that curtailed receptor levels and triggeredatrophy in muscle myotubes but did not influence miR-100 expression. Our results demonstrate modulatory roles of miR-100 in bovine primary muscle cell development, lipid deposition and metabolism. Hence, suggesting a therapeutic role of miR-100 in diabetic muscle and marbling characteristics of meat animals.

P9.11

Despite the evident impact on fibro-adipogenic progenitors differentiation potential, miR-378 deletion does not affect muscle regeneration in a glycerol-induced injury model

Olga Mucha, Paulina Podkalicka, Monika Żukowska, Józef Dulak, Agnieszka Łoboda

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
Olga Jolanta Mucha <olga.mucha@doctoral.uj.edu.pl>

Upon injury, activated fibro-adipogenic progenitors (FAPs) create a fibrotic scaffold supporting muscle regeneration, promote myogenic differentiation and clearance of necrotic debris. miR-378, a small noncoding microRNA, plays an important role in muscle regeneration but its effect on FAPs functioning is unknown.
In our study, we utilized the glycerol-induced injury model, which allows us to study not only regeneration but also fat and fibrous tissue accumulation in the wild type (WT) and miR-378 lacking mice (miR-378$^{-/-}$). The initial histological assessment of the hematoxylin and eosin and Sirius red staining confirmed the correctness of our model showing severe inflammation at days 3 and 7 and fibrous and fat accumulation starting from around day 14. Nevertheless, we did not observe differences between genotypes. Also, the number of centrally nucleated fibers (CNF), calculated on days 7, 14, and 28 was unaffected by the lack of miR-378. No changes were also observed when creatine kinase (CK) activity was measured. Moreover, 28 days after the injury maximal force of the injured muscle was similar to the control (without glycerol injection) animals in both genotypes. More in-depth, FACS analysis performed on day 3, showed a decreased percentage of M2-like macrophages and increased quantity of activated muscle satellite cells in miR-378$^{-/-}$ animals. Importantly, our preliminary data on isolated FAPs shows enhanced adipogenic differentiation potential of miR-378$^{-/-}$ cells both in spontaneous and directional differentiation experiments. Ongoing analyses are focused on the more molecular investigation of FAPs lacking miR-378.

Acknowledgements of Financial Support: This work was supported by grant No. 2019/33/N/NZ4/02885 (OM) from National Science Centre.

P9.12

Phosphorylation of Na^+-K^+ pump at Tyr10 of its α1-subunit is subjected to regulation by AMP-activated protein kinase in cultured myotubes

M. Petrič[1], K. Miš[1], A. Chibalin[2], S. Pirkmajer[1]

[1]Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [2]Department of Molecular Medicine and Surgery, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden
Metka Petrič <metka.petric@mf.uni-lj.si>

Introduction: Na^+-K^+ pump, a heterodimeric (α/β) Na^+,K^+-ATPase (NKA), is essential for skeletal muscle ion homeostasis, excitability, and contractility. As a major metabolic tissue with high demands for ion transport, skeletal muscle requires tight coordination of NKA and energy metabolism. AMP-activated protein kinase (AMPK), a cellular energy sensor, regulates NKA by modulating serine phosphorylation of its α1-subunit.
Aim: Here we examined whether AMPK modulates phosphorylation of the α1-subunit of NKA at Tyr10, an important phosphosite for insulin-induced activation of NKA in the kidney.
Results: AMPK activators AICAR and A-769662 supressed the phosphorylation of Tyr10 of NKAα1 in primary human and rat L6 myotubes. Ouabain, a pharmacological NKA inhibitor and a putative adrenocortical hormone, tended to increase phosphorylation of Tyr10, while PP2, an inhibitor of Src family kinases reduced it. Epidermal growth factor (EGF) stimulated Tyr10 phosphorylation in human myotubes, which was opposed by inhibitors of tyrosine kinases (genistein) and EGF receptor (gefitinib). Tyr10 was not responsive to EGF in L6 myotubes, whose expression of EGF receptor is markedly lower than in human myotubes.
Conclusions: Modulation of phosphorylation of Tyr10, which is conserved in the NKA α1-subunit of multiple vertebrate species, provides a new mechanism by which AMPK regulates NKA. Collectively, our results highlight a link between regulation of NKA and energy metabolism in skeletal muscle.

P9.13

Regulation of Na^+,K^+-ATPase by AMP-activated protein kinase: the strange case of skeletal muscle

Sergej Pirkmajer[1], Metka Petrič[1], Anja Vidović[1], Alexander Chibalin[2]

[1]Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [2]Department of Molecular Medicine and Surgery, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden

Sergej Pirkmajer <sergej.pirkmajer@mf.uni-lj.si>

AMP-activated protein kinase (AMPK) is a cellular energy sensor and regulator of energy metabolism. Under energy-deprived conditions AMPK stimulates ATP production and suppresses its consumption. Na^+,K^+-ATPase (NKA), which consumes 20-25% of ATP in the body, might seem like an ideal target for energy-saving measures. Consistent with its role as a suppressor of ATP consumption, AMPK stimulates endocytosis of NKA in alveolar cells, thus suppressing ion transport in lungs. However, skeletal muscle contractions activate NKA, which opposes rundown of ion gradients, as well as AMPK, which plays an important role in adaptations to exercise. While inhibition of NKA under these conditions would promote loss of excitability and accelerate fatigue, evidence suggests that AMPK does not inhibit or even stimulates NKA in skeletal muscle, which appears to contradict the idea that AMPK always suppresses the ATP consumption. Dephosphorylation of the catalytic NKA α1-subunit at Ser18 was previously shown to be important for AMPK-mediated regulation of NKA in myotubes. We now show that AMPK promotes dephosphorylation of NKA α1-subunit at Tyr10, indicating existence of a new regulatory pathway between AMPK and NKA. Moreover, we show that glucose deprivation not only induces energy stress, but also alters the ratio between the different isoforms of NKA subunits in myotubes. Taken together, these results highlight a close link between regulation of NKA and energy metabolism in skeletal muscle.

P9.14

Biomimetic modulable light-activated contractile unit based on liquid crystalline elastomers

S. Querceto[1], G. Vitale[1], B. Grandinetti[2], D. Martella[2], D. S. Wiersma[2], E. Cerbai[3], C. Tesi[1], C. Poggesi[1], C. Parmeggiani[2], C. Ferrantini[1], L. Sacconi[2]

[1]Department of Experimental and Clinical Medicine, University of Florence, Italy; [2]European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino, Italy; [3]Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence, Italy

Silvia Querceto <silvia.querceto@gmail.com>

Ideally, biocompatible polymers, able to work as actuators, should be able to modulate their strength, kinetics, and stiffness to fit the features of natural muscles. So far, many attempts to create materials or devices have failed in reproducing the natural muscle function and regulation. Our previous mechanical characterization of acrylate-based liquid crystalline elastomers (LCE) highlighted that these biocompatible materials show promising features that may be useful for mimicking muscular function (in terms of active/passive strength and contraction kinetics) and for effectively improving cardiac contraction *in vitro* (Ferrantini et al. 2019). In the present work, we describe the design of a biomimetic contractile unit prototype, built by interposing an ultra-thin LCE film in between two matrixes of eight blue mini-LEDs. The geometry of the mini-LED-LCE prototype was optimized according to the light properties of the LCE in order to maximize the homogeneity of x-y-z illumination. A mechanical characterization of the device was carried out with various light-stimulation protocols and different loading conditions. We show how the LCE contractile performance (in terms of both force amplitude and kinetics) can be fine-tuned by modulating both the individual mini-LED light intensity as well as the number of mini-LEDs in the "ON" state. The force output of the device is closely linked to the illumination pattern and the efficiency optimized if the mini-LEDs in the "ON" state are adjacent. These results pave the way for material scale-up by assembling multiple mini-LED-LCE contractile units and further development of novel LCE-based contraction assist devices for cardiac, skeletal or smooth muscle support or replacement.

P9.15

Evaluation of rat motor activity following spinal cord injury based on motion video analysis

D. E. Sabirova, M. E. Baltin, I. R. Nurlygayanov, V. V. Yaikova, T. V. Baltina, O. A. Sachenkov

Kazan Federal University, Kazan, Russia
Diana Sabirova <sabirova.dianka@list.ru>

In the last 25 years of rehabilitation of patients with spinal cord injury (SCI), the positive effect of physical training on restoring patient mobility was noted. Regardless of the methods used to restore movement, an adequate system is needed to determine changes in coordination and motor ability itself, for this purpose, mainly motion capture systems are used. The aim of this study was to analyze the recovery of motor and postural function in rats after SCI of varying severity and motor training by video motion analysis. The study was carried out in compliance with bioethical norms. Three-dimensional rat gait data were obtained using 4 cameras Vicon MX (Oxford, UK). The angle of flexion of the hind limbs in the joints, the volume of movement of the limb, the height of the foot lift, the lateral deviation of the foot were determined. The results showed that physical training is able to improve the motor function of rats, contributing to the restoration of body weight-supporting locomotion, control of walking direction and the ability to maintain equilibrium in paralyzed rats. The degree of recovery depends on the severity of the SCI. Physical training promotes coordinated operation of postural mechanisms of limb and torso movement regulation and restores initial configuration of body posture when walking in rats.

Acknowledgements of Financial Support: The study was carried out with the financial support of RFFI in the framework of scientific project No. 20-01-00535.

P9.16

Mandibular muscle troponin of the Florida carpenter ant Camponotus floridanus: extending our insights into invertebrate Ca^{2+} regulation

Yun Shi[1], Julia P. Bethea[1], Hannah L. Hetzel-Ebben[1], Maicon Landim-Vieira[2], Ross J. Mayper[1], Regan L. Williams[1], Lauren E. Kessler[1], Amanda M. Ruiz[1], Kathryn Gargiulo[1], Jennifer S. M. Rose[1], Grayson Platt[1], Jose R. Pinto[2], Brian K. Washburn[1], P. Bryant Chase[1*]

[1]Department of Biological Science, Florida State University, Tallahassee, FL 32306 USA; [2]Department of Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL 32306 USA
Yun Shi <ys19f@my.fsu.edu>

Ants use their mandibles for a variety of functions and behaviors. We investigated mandibular muscle structure and function from major workers of the Florida carpenter ant Camponotus floridanus: force-pCa relation and velocity of unloaded shortening of single, permeabilized fibres, primary sequences of troponin subunits (TnC, TnI and TnT) from a mandibular muscle cDNA library, and muscle fibre ultrastructure. From the mechanical measurements, we found Ca^{2+}-sensitivity of isometric force was markedly shifted rightward compared with vertebrate striated muscle. From the troponin sequence results, we identified features that could explain the rightward shift of Ca^{2+}-activation: the N-helix of TnC is effectively absent and three of the four EF-hands of TnC (sites I, II and III) do not adhere to canonical sequence rules for divalent cation binding; two alternatively spliced isoforms of TnI were identified with the alternatively spliced exon occurring in the region of the IT-arm alpha-helical coiled-coil, and the N-terminal extension of TnI may be involved in modulation of regulation, as in mammalian cardiac muscle; and TnT has a Glu-rich C-terminus. From the troponin subunit sequences, a structural homology model was built of *C. floridanus* troponin on the thin filament. From analysis of electron micrographs, we found thick filaments are almost as long as the 6.8 um sarcomeres, have diameter of ~ 16 nm, and typical spacing of ~ 46 nm. These results have implications for the mechanisms by which mandibular muscle fibres perform such a variety of functions, and how the structure of the troponin complex aids in these tasks.

P9.17

Evaluating the potential of nutraceuticals as geroprotectors on skeletal muscle performance

Z. Singlár, P. Szentesi, L. Csernoch, M. Sztretye

Department of Physiology, University of Debrecen, Debrecen, Hungary
Zoltán Singlár <singlar.zoltan@med.unideb.hu>

Skeletal muscle is responsible for posture and movement, along with metabolism and thermogenesis. It comprises approximately 40-45% of total body weight in a 70 kg adult human. Physiological calcium homeostasis is crucial for a well functioning musculature, in which the excitation-contraction coupling (ECC) with its key proteins, the voltage sensor dihidropyridine receptor (DHPR) and calcium channel ryanodin receptor (RyR), along with the mitochondria (acting as a calcium buffer), are proned to be damaged during extensive muscle work and aging processes leading to sarcopenia and frailty.
Astaxanthin, a carotenoid from the xanthophyll group, was shown to exert beneficial effects during strenous exercise and age related muscle degenerations, along with geroprotective effects.
In this work, we show that supplementing young (4-6 months old) and old (>14 months old) C57BL6 mice with astaxanthin (AstaReal A1010, AstaReal Sweden) for 4 weeks in a concentration of 0.02% w.w. can lead to improved *in vivo* muscle force in young mice and improved *in vitro* muscle force for both ages, without inducing any significant changes in the voltage dependency of the ECC mechanism. We also report altered mitochondrial calcium uptake in young, but not old mice, while asserting the activity dependent mitochondrial calcium accumulation in vitro. Our data indicate that astaxanthin supplementation can improve muscle force for young and healthy, and, in old and mice as well with intact calcium homeostasis.

P9.18

Changes in myosin heavy chain expression and capillary network morphology of inspiratory muscles of diabetic individuals

R. T. Tomazin[1], E. Cvetko[1], A. Alibegović[2], J. Janáček[3], N. Umek[1]

[1]Institute of Anatomy, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [2]Institute of Forensic Medicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [3]Department of Biomathematics, Institute of Physiology, Czech Academy of Science, Prague, Czech Republic
Nejc Umek <nejc.umek@mf.uni-lj.si>

Skeletal muscles and the microvasculature play critical roles in the pathophysiology of type 2 diabetes, which is one of the leading causes of morbidity and mortality worldwide. While the functional effect of obesity and diabetes on respiratory muscles has been described, the effect on the morphology of the capillary network and the muscle fibre type composition is less known. The aim of our study was to investigate the diabetes-related changes in the expression of myosin heavy chain isoforms and capillarisation around individual muscle fibres of human inspiratory muscles (diaphragm and external intercostal muscles) using a 3D method. We studied autopsy samples of eight adult males who were obese and had at least 10-year history of type 2 diabetes without complications and eight age-matched non-diabetic lean controls. In the diabetic individuals, the external intercostal muscle was significantly better capillarised and its capillaries were significantly less tortuous and more anisotropic than in the non-diabetic controls. The differences in capillarisation were greater around large muscle fibres (diameter ≥ 45 μm) compared to small muscle fibres. The capillary supply of the diaphragm showed the same trends in all parameters, but the results were not statistically significant. Moreover, we found no significant differences in muscle fibre diameters or muscle fibre type composition between the two studied groups or between the two studied muscles.

P9.19

Effect of glucose on the expression of Na^+,K^+-ATPase subunits in cultured human myotubes

A. Vidović[1], A.V. Chibalin[2], S. Pirkmajer[1]

[1]Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [2]Department of Molecular Medicine and Surgery, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden

Anja Vidović <anja.vidovic@mf.uni-lj.si>

Introduction: Skeletal muscle, which contains the largest pool of Na^+,K^+-ATPase (NKA) in the body, has an important role in ion homeostasis. NKA comprises a catalytic α-subunit (isoforms α1-4) and a glycoprotein β-subunit (isoforms β1-3). In skeletal muscle, FXYD1 or FXYD5 proteins, also known as the γ-subunit, interact with NKA and modulate its function.
Aim: Diabetes mellitus is associated with dysregulation of NKA in skeletal muscle, but the underlying mechanisms remain unclear. Here we determined whether glucose modulates expression of NKA subunits in cultured human myotubes.
Results: As estimated by qPCR, the expression of α1 and α2 subunits was similar (~57% *vs.* 43% of transcripts) under basal conditions (1 g/L glucose), while α3 subunit was at the limit of detection (<0.1% of transcripts). Among the β subunits, β3 and β1 predominated (~79% vs. 20% of transcripts), while levels of β2 were ~1000-fold lower (<1% of transcripts). FXYD1 was less prominently expressed than FXYD5 (18% *vs.* 82% of transcripts). Glucose had most prominent effect on the expression of α1 and α2 subunits, which were upregulated and downregulated, respectively, when glucose concentration was increased from 0 to 4.5 g/L. Mannitol, a metabolically inert osmotic agent, exerted a modulatory effect on their expression.
Conclusions: Glucose modulated the expression of NKA subunits in human myotubes in an isoform-specific manner. The underlying mechanisms likely involve metabolic as well as osmotic effects.

P9.20

Identification of a novel skeletal muscle-specific aptamer to facilitate muscle-targeted drug delivery

Zong-Kang Zhang[1], Ning Zhang[1], Shanshan Yao[1], Hewen Jiang[1], Ge Zhang[2], Bao-Ting Zhang[1]

[1]School of Chinese Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China; [2]Institute for Advancing Translational Medicine in Bone & Joint Diseases, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, China

Zongkang Zhang <maxzhangzk@cuhk.edu.hk>

The skeletal muscular disorders, such as sarcopenia and Duchenne muscular dystrophy, are related to serious health consequences. However, one major bottleneck of current pharmacological therapies for skeletal muscle disorders is lack of muscle cell selectivity, leading to low efficacy and off-target side effects when administered systemically. Nucleic acid aptamers are promising escort molecules for drug delivery systems due to their high affinity and selectivity to target molecules. However, there's still lack of promising skeletal muscle-targeted aptamer. Utilizing cell-based systematic evolution of ligands by exponential enrichment (cell-SELEX) technology, we screened aptamer candidates with high affinity to mouse myoblast C2C12 cells by performing positive selection with C2C12 cells and negative selection with hepatocytes and peripheral blood mononuclear cells. Aptamer SkMApt1 showed higher affinity and selectivity to skeletal muscle than scramble aptamer both *in vitro* and *in vivo*. Moreover, SkMApt1 showed high affinity to human skeletal muscle cells. We previously reported microRNA-487b (miR-487b) is an anabolic suppressor in skeletal muscle during sarcopenia development. To further evaluate the efficacy of SkMApt1-facilitated skeletal muscle-targeted drug delivery, we prepared SkMApt1–functionalized lipid nanoparticles (LNPs) encapsulating antagomiR-487b (Apt1-LNPs-antimiR), which had uniform particle shape, good serum stability, no detectable cytotoxicity and high affinity for muscle cells *in vitro*. The expression level of miR-487b was significantly lower in Apt1-LNPs-antimiR treated C2C12 cells than scramble aptamer-LNPs-antimiR. Taken together, the above findings suggested that SkMApt1 could be a potential skeletal muscle-targeted moiety to facilitate muscle-targeted drug delivery.

Acknowledgements of Financial Support: This study was supported by the Hong Kong General Research Fund (14103420).