Abstract Book

Frontiers in Myogenesis Meeting
Skeletal muscle: development, regeneration and disease
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Program Abstract #1
Muscle stem cell aging and rejuvenating strategies
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Aging is characterized by a progressive decline of physiological integrity leading to the loss of tissue function and vulnerability to disease, but its causes remain poorly understood. Skeletal muscle has an outstanding regenerative capacity that relies on a normally quiescent population of stem cells (satellite cells). This capacity declines with aging, and recent discoveries have redefined our view of how this occurs. We will discuss how an interconnection of extrinsic and cell-intrinsic mechanisms might provoke failure of normal muscle stem cell functions with aging. In particular, we will show that during most part of their lives, these long-lived satellite cells avoid loss of the bona fide quiescent stem-cell state and senescence entry through basal proteostatic systems, in particular autophagy. These protective clean-up systems are lost during aging, resulting in stem cell regenerative decline. Yet, emerging evidence supports the existence of specialized states within the quiescent stem cell population that are maintained throughout most of life. Finally, through a combination of global gene expression/bioinformatics and in vivo assays, we have found that adult quiescent satellite cells are subjected to circadian control, and that they undergo circadian reprogramming with aging. Interestingly, autophagy is one of the intracellular processes that is oscillatory in adult, but not aged, muscle stem cells. Thus, we propose that, through controlling distinct activities, proteostasis maintains muscle stem cell homeostasis and rhythmicity, while its decay is causally implicated in stem cell aging, a process that can be targeted for rejuvenation. This notion connects with the emergent biology of rejuvenation of old stem cells, by restoring traits of youthfulness, with the final goal of preserving and improving human health during aging.

Program Abstract #2
Defining the in vivo dynamics of muscle stem and progenitor cell action during growth and injury
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Live imaging of the collective cellular response to tissue injury remains a long-term goal of the regenerative medicine field. In an attempt to attain this goal we have developed zebrafish models of tissue injury where the optical accessibility of the larvae allows the application of non-invasive techniques to assay repair in real time. Here we apply this approach to regenerating skeletal muscle in order to determine the cellular and molecular events that define regeneration *in vivo*. Transgenic zebrafish reporter lines fluorescently tagging wound-present cellular components were subject to acute injury, enabling the location and response dynamics of individual wound-occupying cells to be correlated to the stem cell compartment during muscle repair. This analysis identified that a specific subset of macrophages 'dwells' within the injury, establishing a transient but obligate stem cell niche required for stem cell proliferation. Single cell profiling identified specific signals secreted from dwelling macrophages that coordinate repair. In contrast to regeneration, organ growth requires a careful balance between cell commitment and stem cell self renewal to maintain tissue growth trajectories. While stem cell action during regeneration and disease has received much attention, the basis of stem cell deployment during organ growth remains poorly defined. Using imaging and fate mapping techniques in zebrafish we identify a lifelong stem cell pool that exhibits extensive clonal drift, shifting from the random deployment of a large population of stem cells during larval growth, to the reliance on a small number of dominant stem cell clones to fuel adult muscle growth. We define a distinct set of molecular mechanisms for the regulation of the stem cells required for organ growth and compare and contrast the regulation of these two distinct muscle stem cell pools.

Program Abstract #3

Inspired by the diaphragm: development of the diaphragm and congenital diaphragmatic hernias

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The diaphragm is a mammalian skeletal muscle essential for respiration and for separating the thoracic and abdominal cavities. Development of the diaphragm requires the coordinated development of muscle, muscle connective tissue, tendon, nerves, and vasculature that derive from different embryonic sources. However, defects in diaphragm development are common and the cause of an often deadly birth defect, Congenital Diaphragmatic Hernia (CDH). We have used mouse lineage and conditional mutagenesis studies to elucidate the developmental origin of the diaphragm and the etiology of CDH. The pleuroperitoneal folds (PPFs), transient pyramidal-shaped structures that form between the thoracic and abdominal cavities, are critical for the early development and morphogenesis of the diaphragm. The PPFs are the target for migrating muscle progenitors and projecting phrenic nerve axons, and the expansion of the PPFs across the surface of the liver guides muscle morphogenesis, formation of the vascular network, and outgrowth of the phrenic nerve. Subsequently, the PPFs give rise to the diaphragm's muscle connective tissue and central tendon. The PPFs are also critical for the etiology of CDH, as mutations in the PPFs are an important source of CDH. We find that CDH-implicated genes are required in the PPFs at the earliest steps of diaphragm development and mutations in these genes cause non cell-autonomous effects on muscle, resulting in muscleless patches. Intriguingly, only muscleless patches with disorganized, and presumably mechanically weakened, connective tissue herniate. Thus we find that PPF-derived connective tissue is critical for trophic and biomechanical support of the developing diaphragm.

Program Abstract #4

Stage-specific effects of Pitx2 in somite-derived muscle development

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During embryonic development, transitory structures called somites give rise to an epithelial dermomyotome, the source of dermal and endothelial precursors, as well as all the skeletal muscles of the trunk. Multipotent muscle progenitor cells (MPCs) that express Pax3 arise from the dermomyotome and acquire their definitive identity via the myogenic regulatory factors (MRFs) Myf5, Mrf4, and MyoD. Moreover, the muscle stem cells (satellite cells) of the body and limbs also arise from somites, in common with the muscle that they are associated with. Several previous evidences have revealed that the transcription factor Pitx2 might be a player within the molecular pathways controlling somite-derived muscle progenitors' fate. However, the hierarchical position occupied by *Pitx2* within the genetic cascade that control somite-derived myogenesis remain unsolved. To get insight into this issue, we have differentially generated two conditional *Pitx2* mutant mice to specifically inactivate Pitx2 in multipotent Pax3+ muscle progenitors (Pax3Cre+/Pitxloxp/loxp mice) and in myogenic committed progenitors (Myf5Cre+/Pitx2loxp/loxp mice). Our analyses revealed that Pitx2 inactivation in Pax3+ precursors lead to impaired myogenesis while the loss of Pitx2 in Myf5+ myogenic cells have an impact in the number of muscle satellite stem cells that reach their niche in the adult muscle with severe consequences in muscle regeneration.

Program Abstract #5

Genetic control of skeletal muscle fiber type

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Skeletal muscles are composed of slow and fast fibers. There are 3 fast fiber subtypes, each expressing a different isoform of myosin heavy chain (Myh) coded by 3 different genes located at the same locus. The spatiotemporal control of the expression of these genes is not known. Our goal is to study the link between the 3D chromatin organization of the Myh locus and the expression of these genes in muscle fibers. We created a transgenic mouse model of the fast Myh locus with a BAC possessing Myh2, Myh1 and Myh4 linked with different fluorescent reporter. This BAC was injected into mouse oocytes and 2

mouse lines were obtained: one line that integrated 2 full copies of the locus (Enh +) and another one with a deletion of the 5' region (Enh-). In the Enh+ line transgenes expression recapitulates that of the endogenous genes. In the Enh- line the 5' region missing seems necessary to spatially control the expression of the genes. By 4C-seq experiments in skeletal muscles, we identified a cis-regulatory module (CRM) that interacts with Myh4 in quadriceps and Myh2 in soleus that corresponds to a super enhancer. Its absence in BAC Enh- mice suggests that this CRM is necessary to activate and restrict the expression of only one Fast Myh gene. Skeletal muscle fibers are plurinucleated cells and less than 5% of fibers express more than one Myh isoform at the protein level. In the other fibers, it is suspected that a single fast Myh of the locus is activated. To test this hypothesis, we visualized the expression of Myh mRNA and premRNA in purified myonuclei by single nucleus RNAseq and by FISH. We observed that only one fast Myh can be expressed in one nucleus and that hybrid fibers express different isoforms at the same time but in distinct nuclei. The denervation of the fast EDL induces an increase of hybrids fibers. This suggesting that the mechanism that leads to the expression of a single fast Myh gene in all the nuclei of an adult myofiber is the innervation.

Program Abstract #6

Mechanisms driving in vivo lineage conversion of vertebrate skeletal muscle into early endoderm-like cells

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The extent to which differentiated cells, while remaining in their native microenvironment, can be reprogrammed to assume a different identity will reveal fundamental insight into cellular plasticity and impact regenerative medicine. To investigate *in vivo* cell lineage potential, we leveraged the zebrafish as a practical vertebrate platform to determine factors and mechanisms necessary to induce differentiated cells of one germ layer to adopt the lineage of another. We discovered that ectopic co-expression of Sox32 and Oct4 in several non-endoderm lineages, including skeletal muscle cells, can specifically trigger an early endoderm genetic program. Endoderm-induced muscle cells rapidly lose muscle gene expression and morphology, while specifically gaining endoderm organogenesis markers via a mechanism resembling normal development. Further, muscle lineage conversion is independent of a dedifferentiation or a pluripotency mechanism, suggesting that reprogramming occurs via direct transdifferentiation. Moreover, loss of Myod and activation of autophagy are necessary for, and can enhance, the earliest events in muscle cell reprogramming, such as nuclei aggregation, revealing that disruption of both the muscle transcriptome and proteome are critical for, and not a consequence of, the initiation of lineage conversion. Our work demonstrates that within a vertebrate animal, differentiated cells can be induced to directly adopt the identity of a completely unrelated cell lineage, while remaining in a distinct microenvironment, suggesting that differentiated cells *in vivo* may be more amenable to lineage conversion than previously appreciated. This discovery of possibly unlimited lineage potential of differentiated cells *in vivo* challenges our understanding of cell lineage restriction and may pave the way towards a vast new *in vivo* supply of replacement cells for degenerative diseases such as diabetes. The studies presented here was funded by the NIH and the Keck Foundation.

Program Abstract #7

Dynamin captures and bundles actin filaments to drive invasive membrane protrusions in myoblast fusion

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Myoblast fusion is critical for skeletal muscle development and regeneration. It is mediated by actin-propelled invasive protrusions generated by the attacking fusion partner and mechanosensitive

responses mounted by the receiving fusion partner. Here we demonstrate a direct and noncanonical role for dynamin, best known as a fission GTPase in endocytosis, in myoblast fusion. Our genetic and cell biological analyses show that dynamin colocalizes within the F-actin-enriched podosome-like structure at the fusogenic synapse and facilitates the generation of invasive membrane protrusions during myoblast fusion in vivo. Biochemical, negative stain EM and cryo-electron tomography (cryo-ET) analyses revealed that dynamin forms helices that directly bundles actin filaments by capturing multiple actin filaments at their outer rim via interactions with dynamin's proline-rich domain. GTP hydrolysis by dynamin triggers disassembly of the dynamin helix, exposes the sides of the actin filaments, promotes dynamic Arp2/3-mediated branched actin polymerization, and generates a mechanically stiff actin network. Thus, dynamin functions as a unique actin-bundling protein that enhances mechanical force generation by the F-actin network in a GTPase-dependent manner. Our findings have general implications for understanding dynamin-actin interactions in various cellular processes beyond myoblast fusion.

Program Abstract #8
Mechanism of myofiber self-repair facing minor injuries
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Skeletal muscle has the capacity to regenerate. This is achieved through a multi-cellular process leading either to the fusion of satellite cells to damaged myofibers or to the generation of entirely new muscle cells. In this study, we explore the capacity of myofibers to self-repair independently of the satellite cell regenerative program. In response to localized injury in fully matured in vitro myofibers, we observed that myonuclei at the periphery of the cell de-anchor and migrate to the injury site. Within 30 hours post-injury, damaged myofibrils are restored and myonuclei depart the repaired area. We identified the molecular mechanism underlying this nuclear movement by investigating the signaling cascade responsible for attracting myonuclei to the injury site, as well as characterizing the molecular machinery responsible for moving the nuclei to the lesion. Moreover, we determined that these nuclear migrations are important to provide de novo mRNAs locally at the injury site as to replenish damaged cellular components such as myofibrils. Blocking nuclear movement to the injury site hampers myofiber self-repair, resulting in delayed restoration or cell death. Myofiber self-repair is a newly discovered mechanism by which muscle cells overcome minor injuries. This process could be a first line of defense against muscle damage and play an important role in muscle homeostasis, viability and disease by either providing time for the satellite cell dependent regenerative program to activate or by limiting the necessity for satellite cell activation thereby preserving the satellite cell pool.

Program Abstract #9
Divergent cell-specific consequences of myomaker expression in dystrophic skeletal muscle
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Fusion of mononucleated myoblasts to form multinucleated myofibers is necessary for the formation of functional skeletal muscle. Myomaker is a muscle-specific membrane protein that is absolutely required for myoblast fusion, and its expression is precisely regulated during muscle development and regeneration. Myomaker is expressed in muscle during the chronic regeneration that characterizes muscular dystrophy (MD), but the impact of its activity on the course of the disease remains unknown. Furthermore, MD is an attractive setting in which to study cellular fusion mechanisms due to chronic, ongoing fusion of myoblasts to myofibers and the formation of new myofibers. Using the *mdx* mouse model of MD, we deleted myomaker in the satellite cell (*mdx*; *Mymk*^{scKO}) or myofiber (*mdx*; *Mymk*^{fiberKO}) compartments using tamoxifen-inducible CreER alleles. Five months post-tamoxifen administration, *mdx*; *Mymk*^{scKO} displayed a total lack of fusion, resulting in severe muscle loss, enhanced fibrosis, and significant functional decline. *Mdx*; *Mymk*^{fiberKO} mice, however, displayed no alteration of fusion dynamics and showed preservation of *de novo* myofiber formation. Unexpectedly, we observed an improved phenotype in *mdx*; *Mymk*^{fiberKO} mice, with enhanced function and decreased muscle damage, assessed by serum creatine kinase and IgM immunostaining. Taken together, our data indicate that myomaker has divergent effects on dystrophic disease severity depending upon its compartment of expression. Muscle

cell fusion, dependent upon myoblast expression of myomaker, is absolutely required for effective regeneration in muscular dystrophy. However, myomaker expression in dystrophic myofibers, as a consequence of the chronic regenerative process, is not only dispensable for fusion but also detrimental to the integrity of the muscle fiber membrane. These data provide insight into mechanisms of myoblast fusion *in vivo* and reveal a novel aspect of disease pathogenesis of muscular dystrophy.

Program Abstract #10
Nuclear positioning during skeletal muscle development
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Nuclear movements are important for multiple cellular functions. During skeletal myofiber formation or regeneration, nuclei move from the center to the periphery of the myofiber for proper muscle function. In different muscle disorders centrally located nuclei are found. We demonstrate that nuclear movement to the periphery of myofibers is mediated by centripetal forces around the nucleus generated by myofibrils to squeeze the nucleus to the periphery of myofibers. In addition, an Arp2/3 complex containing Arpc5L together with γ-actin is required to organize desmin to cross-link myofibrils for nuclear movement. We also show that peripheral nuclear positioning is triggered by local accumulation of fibronectin secreted by myofibroblasts. We demonstrate that fibronectin via α5-integrin mediates peripheral nuclear positioning dependent on FAK and Src activation. Thus we identify that local activation of integrin by fibronectin secreted by myofibroblasts activates peripheral nuclear positioning in skeletal myofibers.

Program Abstract #11
Regulation of muscle stem cells quiescence and activation
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Tissue resident stem cells are maintained quiescent in specialized niches and are capable to repair an organ following injury. Skeletal muscle stem cells, known as muscle satellite cells, are the indispensable cell population responsible of skeletal muscle homeostasis and regeneration in response to injury. In resting condition, the muscle satellite cells are quiescent, and upon niche disruption they rapidly exit quiescence towards an activated proliferative state. However, despite the identification of several molecular regulators of muscle stem cell quiescence and activation, the actual gene regulatory network regulating the initial transition between these cell states remains largely unknown. We implemented a fixation-based protocol to capture cells in their native state, and generated a high-resolution transcriptional map of muscle satellite cells early activation. By time-course analysis, we have captured the earliest transcriptional responses of *in vivo* quiescent stem cells, and uncovered kinetically co-regulated genetic modules that define a precise sequence of cellular processes that drive cells out of quiescence. Moreover, we found that in response to muscle injury, individual muscle stem cells react asynchronously yet follow a unique activation trajectory. Overall, our study proposes a mechanism of quiescence exit that obeys a precise series of biological function, whereby early proliferation signals act independently of the myogenic signals that occur later. In addition, we identified a muscle stem cell-specific function for the histone chaperone HIRA and Daxx that regulate the deposition of the histone H3 variant H3.3 during replication-independent nucleosome assembly that will be discussed during my presentation.

Program Abstract #12
Post-transcriptional regulation of quiescence exit
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Quiescent adult stem cells have the ability to respond rapidly to external stimuli, but mechanisms of such rapid activation remain elusive. Using an in vivo fixation approach, we have recently revealed a widespread intron retention phenomenon in polyadenylated mRNAs isolated from quiescent muscle stem cells. Genes possessing IR are essential for various fundamental cellular functions including RNA splicing, protein translation, cell cycle entry and lineage specification. Further analysis revealed that IR is a post-transcriptional mechanism that regulates QSC quiescence exit, which is dependent on the phosphorylated-Dek protein. While Dek is absent in QSCs, overexpression of Dek in QSC in vivo results

in a global decrease of IR, quiescence exit, and consequently undermine muscle regeneration. Moreover, IR analysis on public RNA-seq data shows that other quiescent adult stem cells are enriched with retained introns, indicating IR as a feature of quiescent adult stem cells. Altogether, these findings suggest that intron retention plays an important role in stem cell quiescence exit.

Program Abstract #13
The adhesion G-protein coupled receptor GPR116 is required for muscle stem cell quiescence and self-renewal
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Regeneration of skeletal muscle largely depends on a resident pool of quiescent muscle stem cells (MuSCs). In response to muscle injury, MuSCs activate the myogenic program and enter the cell cycle in order to amplify a population of myogenic progenitors that either differentiate into new myofibers, or undergo self-renewal to maintain the MuSC pool. The MuSC microenvironment, or 'niche' is critical for maintaining the MuSC pool, however how signals are relayed from the niche to MuSCs is less clear. MuSCs express high levels of *Gpr116*, a member of the adhesion G-protein coupled receptor (GPCR) family that are named for their large N-terminal adhesive domains capable of mediating cell-extracellular matrix (ECM) interactions. Here, we show *Gpr116* is required for MuSC quiescence, self-renewal and long-term maintenance of the MuSC pool. Conditional inactivation of *Gpr116* in MuSCs leads to impaired self-renewal, with consequent muscle regeneration defects after repeated injury. Moreover, MuSC specific *Gpr116* ablation in the *mdx* mouse model of Duchenne muscular dystrophy leads to progressive loss of the MuSC pool, impaired muscle regeneration and decreased force generation. In bioluminescence resonance energy transfer (BRET) assays, stimulation of GPR116 with its tethered peptide agonist leads to strong coupling with β-arrestin, compared to weak coupling with Gα protein. We propose a model by which GPR116 receptor activation initiates β-arrestin signaling to maintain the MuSC pool. Our results highlight for the first time the role of an adhesion GPCR regulating adult stem cell quiescence and self-renewal, potentially mediated via a novel β-arrestin dependent signaling pathway.

Program Abstract #14
Muscle stem cell heterogeneity and the niche
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Pax7-expressing satellite cells (SCs) function as an essential stem cell population capable of extensive self-renewal and skeletal muscle repair. In response to muscle injury, SCs exit quiescence, transition towards cell cycle entry, leading to replication, migration to the injury site and either self-renewal or differentiation. SCs are functionally heterogeneous with subsets biased to self-renew (stem cells) or differentiate (committed progenitors). In this presentation I will discuss our studies that uncover molecular and functional heterogeneity within the SC pool and the molecular programs that maintain SCs in a quiescent state within the niche.

Program Abstract #15
Spatial mapping of muscle regeneration at the single cell level using CODEX
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Skeletal muscle regeneration requires an orchestrated response from a variety of cell types that promote the repair process. Changes in the cellular composition and their secreted cytokines are necessary to instruct muscle stem cells to expand and differentiate to replace damaged tissue. Dysregulation of cellular response (e.g. ablation of support cell types) or signaling (e.g. aberrant growth factor expression) is sufficient to perturb the regeneration process. Moreover, it is a central hypothesis that dysregulated

responses during recovery in disease states can trigger fibrosis and ineffective repair. Thus, a deeper understanding of the cellular interactions during regeneration and spatial-temporal relationships between stem cells and support cells has significant implications for disease progression and can lead to the identification of targets for regenerative medicine approaches. A systematic approach to quantify cellular relationships at the single cell level and methods to comprehensively map cellular crosstalk has been limited by the lack of detailed spatial information at the single cell resolution. Here, we utilize CODEX, a potent multiparametric imaging technique, which enables simultaneous visualization of 40 antibodies in tissues, to overcome this limit to study cellular dynamics during muscle regeneration *in situ*. By mapping the spatial dynamics of 27 cell types (stem cells, immune cells, and stromal cells) during regeneration, we reveal the critical role of the immune response in tissue repair and quantify interactions between muscle stem cells and support cell types. This has allowed us to identify physiologically relevant cell-cell interactions that drive regeneration and unmask previously undescribed disease mechanisms.

Program Abstract #16
Selective mRNA Translation Regulates Satellite Cell Activity
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Regeneration of adult tissues depends on somatic stem cells that remain quiescent, yet are primed to enter a differentiation program. Growing evidence indicates that these properties are regulated in part by maintaining low levels of protein synthesis in quiescent adult stem cells, but it remains unclear whether 'selective' mRNA translation defines stem cell properties. We show that a general repression of translation, mediated by the phosphorylation of translation initiation factor eIF2α at serine 51 (P-eIF2α), is required for muscle stem cell (MuSC) quiescence and self-renewal. Skeletal muscle stem cells unable to phosphorylate eIF2α exit quiescence, activate the myogenic program and differentiate, but do not self-renew. Pharmacological inhibition of eIF2α dephosphorylation permits *ex vivo* expansion of MuSCs that retain regenerative capacity after engraftment into the *mdx* mouse model of Duchenne muscular dystrophy. While eIF2α phosphorylation leads to a general repression of protein synthesis, specific mRNAs are selectively translated in a P-eIF2α dependent manner. In MuSCs, we show that the mRNA for centrosome/spindle apparatus associated protein TACC3 is selectively translated in a P-eIF2α dependent manner. MuSCs deficient for *Tacc3* activate the myogenic program, but are defective in both proliferation and self-renewal, leading to defects in both regeneration of muscle and restoration of the MuSC pool. We propose a model whereby P-eIF2α ensures in part the robust translational silencing of accumulating mRNAs that is needed to prevent the activation and subsequent differentiation of MuSCs while, on the other hand, allows selective translation of specific mRNAs needed for MuSC expansion and self-renewal.

Program Abstract #17
Looking under the hood of satellite cell fate control: mechanisms underlying the interplay between ubiquitin ligase Nedd4 and deubiquitinase USP7 in muscle progenitors.
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Satellite cells (SCs) are critical for muscle regeneration and long-term maintenance, thus understanding how muscle formation and SC renewal are balanced is the focus of intense research. Previous work indicates that coordinated post-translational control of Pax7 and the muscle regulatory factors (MRFs) regulates SC fate. The ubiquitin-proteasome system (UPS) plays a critical role in this context, since Pax7 up-regulation induces degradation of the MRF MyoD via the UPS, while induction of muscle differentiation requires UPS-dependent Pax7 decline. Our unpublished data indicate that myogenesis also requires Usp7 deubiquitinase to stabilize Myogenin protein, allowing terminal differentiation. Ongoing work indicates that both Nedd4-1 and Usp7 regulate additional steps in myogenic progression, distinct from the control of Pax7 and MRF protein stability. Specifically, SC-specific deletion of *nedd4-1*impairs muscle regeneration, resulting in a ~50% muscle mass reduction. Interestingly, lineage tracing analyses show that, while Nedd4-null SCs fail to form myotubes in vitro, they form myofibers with a reduced diameter *in vivo*. Using primary myoblast cultures and the C2C12 cell line, we present evidence indicating that Nedd4-1 is involved in the regulation of mitochondrial content and/or remodeling. Specific changes in

mitochondrial content, morphology, and function are required for muscle differentiation, while dysfunctional mitochondria trigger catabolic signaling pathways which ultimately lead to muscle atrophy. In this context, we hypothesize that SCs which overcome the initial barrier imposed by Nedd4-1 loss give rise to “atrophied” myofibers in vivo, revealing an additional requirement for Nedd4-1 during adult myogenesis. Current efforts are directed i) to analyze global changes in protein ubiquitination upon Nedd4-1 loss and ii) to study mitochondrial content and morphology in myofibers derived from Nedd4-null SCs *in vivo* (Supported by FONDECYT#1170975 to HO).

Program Abstract #18
Role of STAT3-mediated autophagy in driving muscle regeneration during aging
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Age-related neuromuscular diseases are associated with a decline in muscle stem cell (MSC) function and an age-dependent muscle wasting, also called sarcopenia. It has been demonstrated a key role of STAT3 in regulating MSC expansion and differentiation, extending its use as therapeutic target to ameliorate muscle wasting. In addition, we recently described the essential role of autophagy in driving MSC function toward efficient muscle regeneration. The established role of autophagy in maintaining muscle mass and tissue homeostasis together with the emerging role of STAT3 in regulating the autophagic process inspired the rationale behind this work which resides in the study of the autophagic contribution in mediating STAT3 signalling toward skeletal muscle repair, a function that is compromised during aging. Our hypothesis is that STAT3 might have a role in regulating myogenic lineage and regeneration process by affecting the autophagic process thereby restoring the pro-myogenic niche that support muscle regeneration. We show that the autophagic process influences MSC activation and proliferation suggesting that autophagy might exert different functions depending on MSC proliferative vs. myogenic state and in muscle fibers. Likely, a combination of autophagy impairment in both compartments cooperates in muscle wasting during sarcopenia.Our further analysis indicates that STAT3 displays nuclear localization in conditions of active autophagy –i.e. young mice- while STAT3 localize in the cytoplasm in aged muscles, characterized by reduced autophagic process.Altogether, these evidences suggest that the nuclear/cytoplasmic compartmentalization of STAT3 regulates the autophagic process and the regenerative drive, highlighting potential biological targets that preclude an efficient regenerative response in aged mice. Funding: Italian Ministry of Health n. PE-2016-02363049 to L.L.

Program Abstract #19
Rejuvenating Stem Cell Function to Increase Muscle Strength
Helen M. Blau
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Regenerative medicine holds great promise for local enhancement of skeletal muscle repair to treat muscular dystrophies and aging-associated muscle wasting. Muscle stem cells (MuSCs) are a potent population that resides within muscle tissues, poised to repair muscle damage throughout life. However, the therapeutic utility of MuSCs is currently limited by their rarity and their inefficient survival, self-renewal, and differentiation after injection into muscle tissue. We have devised bioengineering strategies and discovered novel molecular regulators to surmount these hurdles. By defining the myogenic stem cell progression by single cell mass cytometry (CyTOF), we can target metabolic functions that dictate cell fate transitions. By fabricating biomimetic hydrogels with differing elasticity matching muscle tissue, we can overcome the loss of stem cells on traditional plastic cultureware. Fibrosis, which causes dysfunction and ultimate failure of numerous tissues with aging, is characterized by increased tissue stiffness. We have developed a dynamic hydrogel platform to enable mechanistic studies of cellular dysfunction as fibrosis progresses in real time. Cell autonomous defects in MuSC function accompany aging. By targeting these molecular pathways, we can rejuvenate stem cell function. As an alternative to cell therapy, we are seeking to stimulate the function of endogenous quiescent satellite stem cells within muscle tissues. Through an in silico screen, we identified a potent regulator that robustly augments stem cell function and may serve as a novel therapeutic agent to induce muscle regeneration and counter debilitating muscle wasting in the elderly.

Program Abstract #20

Single-nucleus transcriptomics of the skeletal muscle reveals functional compartmentalization in syncytial cells and its disruption in a dystrophy model

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Nuclei of multi-nucleated skeletal muscle cells are functionally distinct to meet the needs for gene expression at specific compartments of the large syncytium. However, the heterogeneity among myonuclei has not been systemically investigated before. To this date, studying gene expression in the myofiber has relied on either probing selected candidates by RNA in situ hybridization or profiling the entire muscle tissue. The first approach is difficult to scale up to large number, whereas the latter averages out the transcriptomes of all nuclei. To overcome these limitations, we performed single nucleus RNA-sequencing from isolated myonuclei of resting and regenerating muscle, and from muscle of the *Mdx* dystrophy model. Our results revealed a heterogeneity that is not explained by fiber-type differences. We detected nuclei located at the neuromuscular junction, the best documented compartment of the muscle, and compartments at the myotendinous junctions. Interestingly, we found two distinct populations in this region of the muscle, which are enriched in resting and regenerating muscle, respectively. Analysis of the *Mdx* muscle showed huge alterations of the overall transcriptomic profile as well as the composition of nuclear heterogeneity. In sum, our study offers new insights into how the architecture of a multi-nucleated muscle cell is organized, and provides a rich resource for future studies. Our approach should be also applicable to other multi-nucleated cells.

Program Abstract #21

Positional memory governs satellite cell function in adult muscles

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Skeletal muscle is a contractile tissue that is distributed throughout the body, with functionally heterogeneous properties among different muscles. Recent studies have revealed functional heterogeneity in the satellite cells of different muscles, dependent not only on fiber-types but also on regional identity (herein we call it “positional memory”). We investigated 1) when positional memory is established, 2) how it is maintained, and 3) what function(s) it fulfills in adult muscle. Genome-wide gene expression analysis in adult muscles throughout the body revealed that distribution of homeobox (Hox) gene (master regulators of the animal body-plan that guide morphogenesis during development) expression is regionally specific and mirrors developmental origins. Thus, it may serve as a fine molecular signature for positional memory in adults. Expression of Hox genes is epigenetically and robustly maintained in the muscle and its associated satellite cells in adult mice and humans. We showed that Hoxa10 expression, distributed mainly in limb muscles, plays an indispensable role in adult muscle regeneration by regulating genomic stability in satellite cells. Although postnatal deletion of Hoxa10 in satellite cells resulted in a remarkable decline in regenerative ability in $Pax7^{CreERT2/+};Hoxa10^{f/f}$ adult mice, the function of satellite cells in $MyoD^{Cre/+};Hoxa10^{f/f}$ adult mice was unaffected by genetic loss of Hoxa10 in muscle progenitors at developmental stage. Our results suggest that Hox-dependent positional memory is flexibly established during development and governs satellite cell function once it is fixed, potentially influencing region-specific weakness in specific muscle diseases.

Program Abstract #22

EphA7 promotes myogenic differentiation via cell-cell contact

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Skeletal muscle is unique among tissues in adult vertebrates in that each cell is a syncytium of hundreds or thousands of myonuclei, formed by the fusion of terminally-differentiated myocytes. The conversion of proliferating skeletal muscle precursors (myoblasts) to myocytes is a critical step in skeletal muscle development and repair which must be precisely regulated to prevent either proliferation at the expense of differentiation or precocious differentiation, both of which will result in suboptimal numbers of functional myocytes. The syncytial nature of muscle fibers adds another requirement in that differentiation should

only happen when there is a sufficient number of other myocytes nearby to fuse with: a single myocyte (or many single myocytes) is not useful *in vivo*. With this in mind, the common observation that myogenic cells differentiate poorly (regardless of serum conditions) when cultured a low density and will differentiate *en masse* (again regardless of serum conditions) when cultured at high density is not surprising. We have recently identified EphA7 (a member of the Eph family of receptor tyrosine kinases which participate in contact-mediated, bidirectional signaling when bound to their ligands, ephrins) which: 1. is expressed by all and only terminally-differentiated myocytes and immature myofibers during development and regeneration; 2. is necessary for density-dependent differentiation *in vitro* and for muscle hypertrophy *in vivo*; and 3. promotes myogenic differentiation *in vitro* in a cell-nonautonomous fashion. EphA7 has previously been implicated in multiple developmental processes including cranial neural tube closure and axon repulsion and mapping; in these contexts it signals in reverse (the ephrin 'ligand' is expressed by the receiving cell) via ephrin-A5. We therefore propose that EphA7 is a mediator of the community effect in skeletal muscle, and that it acts by signaling in reverse to ephrin-A5-expressing myoblasts.

Program Abstract #23
Ciliary Hedgehog signaling controls fatty fibrosis and muscle repair
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The Hedgehog (Hh) pathway signals through the primary cilium to regulate many diverse functions during development. However, the role of ciliary Hedgehog signaling in mature tissues is less well understood. Skeletal muscle has a remarkable capacity to regenerate. However, in chronic diseases such as Duchenne muscular dystrophy (DMD) or with age healthy muscle is replaced by fibrotic scar tissue and adipocytes, eventually leading to muscle failure. Although Hedgehog signaling can improve muscle function in old and diseased muscle, it is unclear which Hh ligand is being secreted and which cell type responds to it. We discovered that desert hedgehog, DHH, is the main Hh ligand through which the Hh pathway is being activated in skeletal muscle. As Hh signaling functions through cilia, we also surveyed which cell types in skeletal muscle possess a primary cilium and could, therefore, respond to DHH. We discovered that a mesenchymal stem cell population called fibro/adipogenic progenitors (FAPs) dynamically upregulate ciliation during regeneration and give rise to adipocytes after injury. Removing cilia from FAPs impaired their ability to differentiate into adipocytes. Interestingly, loss of cilia also improved muscle repair after an acute injury and prevented muscle decline in a mouse model of DMD. Our results indicate a post-developmental function for ciliary Hh signaling in controlling the response to muscle injury and point to a strategy to combat fatty degeneration of skeletal muscle.

Program Abstract #24
Promoter-proximal stalling of RNA-Polymerase II is required for repopulation of the satellite cell niche after muscle injury
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Efficient regeneration of adult skeletal muscle is mediated by satellite cells which must undergo co-ordinated changes in gene expression to mediate cell fate transitions that generate terminally differentiated myofibers. While RNA Polymerase II (RNA Pol II) stalling has been shown to occur in development to permit rapid changes in gene expression, the role for this transcriptional regulatory mechanism in satellite cell mediated muscle regeneration has not been explored. To examine the role of RNA Pol II stalling in muscle regeneration, we generate mice with an inducible, satellite cell-specific knockout of the NELF-B gene (NELF scKO). Using the cardiotoxin muscle injury model, NELF scKO mice showed impaired skeletal muscle regeneration at 28 days after tibialis anterior muscle injury, as characterized by decreased myofiber size and reduced satellite cell numbers. As a result of these reduced satellite cell numbers, the TA muscle could not be regenerated after consecutive muscular injuries. *Ex vivo* experiments showed that satellite cells from the NELF scKO mice were highly efficient at undergoing terminal differentiation to form myotubes. In contrast, both *in vivo* and *ex vivo* studies of satellite cells from NELF scKO mice experiments showed reduced proliferation due to a propensity to undergo premature differentiation. The mechanisms that cause NELF scKO satellite cells to move forward through cell fate transitions has been explored using bulk RNA-Seq, single-cell RNA-sequencing, and precision run-on sequencing (PRO-seq) experiments. Results of these studies will be discussed in the

presentation. Collectively, our results show that NELF-B induced promoter-proximal pausing of RNA Pol II is required for efficient expansion of satellite cells to allow for proper myofiber formation, and for the undifferentiated satellite cells to repopulate the niche on newly formed myofibers.

Program Abstract #25

H3K9 methylation controls Fibro-Adipogenic Progenitors identity and skeletal muscle repair.

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Fibro-Adipogenic Progenitors (FAPs) are crucial regulators of muscle homeostasis as they possess the intrinsic ability to either support muscle regeneration or to contribute to fibro-adipogenic degeneration of dystrophic muscles. Thus, the elucidation of the molecular mechanisms controlling their phenotypical plasticity holds therapeutic potential. Here, we provide evidence that FAPs are particularly enriched in histone H3 lysine K9 methyltransferases (H3K9 KMTs), G9a and GLP. Our data support a role for G9a/GLP in preserving FAPs identity by repression of alternative transcriptional programs through deposition of H3K9 di-methylation (H3K9me2). We show that H3K9me2 is specifically restricted to a layer of peripheral heterochromatin in FAPs, where we found confined the genomic loci of master myogenic genes, such as *MyoD*. Moreover, we identified Prdm16, an H3K9 KMT required for heterochromatin integrity, as a FAP-specific nuclear lamina (NL)-enriched factor, that controls G9a/GLP-dependent H3K9me2 deposition at myogenic loci. Of note, *in vivo* FAP-specific ablation of Prdm16 promotes acquisition of a myogenic fate, thus enhancing skeletal muscle repair. These data are further corroborated by the evidence that FAPs of PDGFRa-H2A::eGFP mice treated with G9a/GLP specific inhibitors are able to participate to myogenesis *in vivo*. Together, our findings reveal a FAPs-specific epigenetic axis important to control their identity. This pathway is also of therapeutic relevance since we demonstrate that *in vivo* inhibition of H3K9 methylation in dystrophic mice enhances skeletal muscle regeneration, inducing an increase in myofibers' size and reduction of adipogenic and fibrotic scars.

Program Abstract #26

The Polycomb group protein Ezh1 regulates skeletal muscle stem cell quiescence and regenerative potential

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Skeletal muscle stem cells (MuSCs) remain quiescent under normal physiological conditions ensuring maintenance of the stem cell pool. Although the molecular regulation of MuSC quiescence has been intensively studied, little is known about the role of chromatin and epigenetic modifications in this process. Here we show that Ezh1, a H3K27 methyltransferase of the Polycomb Repressive Complex-2 (PRC2), maintains MuSC quiescence by regulating expression of core components of MuSCs including those of the Notch pathway. Chromatin of Ezh1-null MuSCs is more accessible leading to increased expression of MyoD and precocious activation of late muscle differentiation genes. Genetic deletion of Ezh1 promotes untimely activation of MuSCs, leading to impaired muscle regeneration and depletion of stem cell reservoir upon consecutive injuries. Together, these findings suggest that Ezh1 safeguards MuSC quiescence by maintaining proper chromatin organization to prevent early activation while promoting transcriptional activation of genes important for MuSC homeostasis.

Program Abstract #27

MYOD-directed re-wiring of 3D chromatin architecture during nuclear reprogramming toward skeletal myogenesis

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Growing evidence indicates the importance of the three-dimensional (3D) genome organization for the spatiotemporal regulation of gene expression. In particular, recent studies have revealed that the genome is folded into a hierarchy of chromatin domains that are defined by high-order chromatin interactions, which spatially and dynamically constrain interactions between regulatory elements and genes. The dynamic nature of high-order chromatin interactions and their relationship with changes in the expression of genes implicated in the control of cell identity, lineage specification and terminal differentiation, prompted our interested to investigate the potential relationship between, MYOD, the master activator of skeletal myogenesis, and changes in high-order chromatin interactions during commitment to the myogenic lineage and differentiation in skeletal myofibers. Genome-wide, high-resolution, Chromatin Conformation Capture technology (Hi-C and 3C), integrated with ChIPseq and RNAseq analysis, revealed that MYOD directs extensive re-wiring of chromatin interactions involving cis-regulatory and structural genomic elements, including promoters, enhancers and insulated neighborhoods (INs). Re-configured INs were hot-spots of differential interactions, whereby MYOD binding to highly constrained sequences of IN boundaries and/or inside INs leads to alterations of promoter-enhancer interactions to repress cell-of-origin genes and to activate muscle-specific genes. Functional evidence, using dCAS9-mediated blockade of MYOD binding, revealed the requirement for MYOD/ DNA binding to re-wire chromatin interactions and regulate local gene expression. Finally, a time course analysis showed MYOD-directed re-wiring of chromatin interactions temporally preceded transcriptional activation of target genes.These data illustrate a model whereby MYOD alters multi-loop hubs, enabling coordinated repression of cell of origin gene networks and activation of tissue-specific genes.

Program Abstract #28
iPS cells: potential for cell therapy, disease modeling and drug discovery in muscular dystrophies
Rita Perlingeiro
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Pluripotent stem cell-derived myogenic progenitors represent an attractive cell source for regenerative medicine, disease modeling, and drug screening of muscular dystrophies (MD). During this talk, I will be discussing our recent findings on the gene editing of Limb-Girdle MD patient-specific iPS cells, the key aspect of *in vitro* maturation for proper disease modeling of neuromuscular disorders, and our recent efforts in developing a cGMP protocol for the manufacturing of a clinically relevant pluripotent stem cell-derived myogenic progenitor cell product.

Program Abstract #29
In vitro modeling of human muscle development and disease
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Progress toward finding a cure for muscle diseases has been slow because of the absence of relevant cellular models and the lack of a reliable source of muscle progenitors for biomedical investigation. We have developed an optimized serum-free differentiation protocol to efficiently produce striated, millimeter-long muscle fibers together with satellite-like cells from human pluripotent stem cells (ES/iPS) *in vitro*. By mimicking key signaling events leading to muscle formation in the embryo, this directed differentiation protocol recapitulates the developmental sequence of myogenesis and avoids the requirement for genetic modifications or cell sorting. We engineered a series of human iPS reporter lines including PAX7-YFP, MYOG-YFP and PAX7-YFP/MYOD1-Cherry that we used to characterize the differentiation of the myogenic lineage in human myogenic cultures. We are particularly interested in the ontogeny of the human PAX7-expressing lineage that leads to the adult satellite cells. We show that human PAX7-YFP cells produced in vitro exhibit characteristics of fetal satellite cells. We performed single cell RNA sequencing of the PAX7-YFP cells generated in vitro and identified the major steps of the differentiation of this lineage. This work provides a framework to study early stages of human myogenesis which are poorly accessible in the embryo.

Program Abstract #30

Reporter hESCs for PAX7 and MYF5 provide valuable insights into myogenic differentiation of hPSCs as well as transcriptional regulation of skeletal myogenesis

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To study myogenic differentiation of hPSCs, a double myogenic reporter hESC (H9) cell line for PAX7 and MYF7 was generated using CRISPR/Cas9n. This system allowed bicistronic expression of the bright fluorescent reporters (tdTomato for PAX7 and EGFP for MYF5). Then by using pathway chemical screen, a short-term yet efficient myogenic induction method was defined, allowing derivation of myogenic progenitors from hPSCs in 2 weeks. In addition, surface marker profiling allowed identification of new markers for enrichment of myogenic progenitors and exclusion of unwanted cells. *In vivo* experiments confirmed myogenic ability of the hPSC-derived cells by engraftment into NSG-mdx mice, dystrophin restoration and satellite cell seeding. This strategy was further validated in healthy as well as patient-derived iPSCs for myogenic differentiation and disease modeling. In addition, modification of the reporter cells using a synergistic activation mediator (SAM), containing a newer dCas9-activator system (sgRNA MS2- incorporating two MS2 RNA aptamers at the tetra-loop and stem-loop, NLS-dCas9-VP64 and MS2-P65-HSF1) allowed augmented activator sensitivity and robust single sgRNA-mediated gene upregulation, as confirmed by flow cytometry and gene expression. This amplified sensitivity enabled performing a genome- wide gain of function screen using a sgRNA activator library containing more than 70,000 sgRNA activator, targeting every gene coding isoform in the human genome. Reporter-positive cells have been purified and sequenced to identify corresponding integrated sgRNAs and thus their corresponding genes. Top candidate activator gene lists for PAX7 and MYF5 are currently being studied using single sgRNA activation and cDNA over-expression experiments to prove their role in activation of myogenic program. These studies are funded by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) of the NIH under award numbers 1R01AR068293 and 1R21AR071583.

Program Abstract #31

iPSC-induced skeletal myoblast lineages for disease modeling and therapeutics

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We have developed a protocol for the efficient isolation of induced secondary myoblast (iSM) lines that can be propagated from cultures of patient-derived iPSCs (induced pluripotent stem cells) responding to growth factor induction of skeletal myogenesis. The growth and differentiation phenotypes of iSMs derived from heathy control, FSHD, LGMD 2G and FKRP dystroglycanopathy iPSCs have been compared to iPSC-induced primary myogenic cells (iPMs) and adult biopsy-derived myogenic cells using flow cytometry, immunohistology, single cell RNAseq and transcriptome analyses to define their lineage relationships and their differentiation gene expression programs, both in cell culture and in muscle xenografts in NSG immune deficient mice. iSMs provide unique ex vivo and in vivo models of human muscle diseases. iSMs derived from iPSCs of patients with infantile- and adult-onset FSHD have enabled investigations of the developmental mechanisms underlying the molecular pathology and epigenetic dysregulation of the DUX4 FSHD disease gene contributing to disease pathology, onset and severity. iSMs produced from iPSCs of an LGMD 2G patient have enabled development of a Cas9 single-cut gene therapy for the efficient and precise therapeutic correction of disease-causing TCAP microduplication mutation. Successful microduplication correction has been accomplished in iPSC-derived LGMD 2G skeletal muscle and cardiomyocytes. In addition to LGMD 2G, we have identified 144 other skeletal

muscle, cardiac and neural diseases, including DOC7 and LAMA2 muscular dystrophies and Tay-Sach's disease, with microduplications treatable by single-cut Cas9 therapeutics (Nature (2019) 568:561).
Support: Muscular Dystrophy Association; LGMD 2i Fund; the UMass Wellstone MDCRC; NIH

Program Abstract #32
Skeletal Muscle Niche Dynamics in Development and Disease
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We are using human pluripotent stem cells to generate skeletal muscle progenitor cells (hPSC-SMPCs) for understanding human myogenesis and development of cell therapies. My previous work showed that increased myogenic ability resides in the ERBB3+NGFR+ fraction of hPSC-SMPCs. We developed a single cell RNA-Seq atlas of human PAX7+ cells across fetal, juvenile and adult which identifies hPSC-SMPCs align between human fetal weeks 8-12 using diffusion map analysis. PAX7+ cells fulfill different functional needs during myogenic development. We show human fetal SMPCs and adult satellite cells (SCs) differ in their ability to make myofibers *in vitro* and *in vivo*; and fetal SMPCs have reduced ability to home to mouse SC niches compared to adult SCs upon engraftment. We have shown hPSC-SMPCs engraft to restore new myofibers similar to levels seen by fetal SMPCs. We also found hPSC-SMPCs fuse to form hundreds small human-only myofibers *in vivo*. PAX7+ hPSC-SMPCs were primarily associated with these regenerating human-only myofibers. We found human-only myofibers continue to grow over 60 days *in vivo*, and PAX7+ hPSC-SMPCs associate under the basal lamina of emerging myofibers over time. This work demonstrates for the first time that fetal and hPSC-SMPCs can be used as a model to study human myofiber formation and niche occupancy *in vivo*. Using mdx-NSG Pax7-cre DTA mice, we evaluated whether population-specific ablation of mouse Pax7 SCs would improve retention of engrafted human PAX7 cells. We found an increase in human PAX7 cells in mdx-NSG PAX7 DTA mice in human niches but not mouse SC niches. Our work suggests that donor cell niche homing occurs in newly regenerating fibers, and not to empty SC niches of established myofibers. Evaluating human niche formation over time will improve our understanding of how human muscle SC niches develop. This could improve our ability to generate *de novo* human niches and better support human PAX7 cells *in vivo* for cell therapy.

Program Abstract #33
Human Duchenne Muscular Dystrophy iPSC-derived myotubes as an in vitro model for studying epigenetic alterations controlling the fibrotic response: role of the epigenetic regulator SETDB1
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The development of therapeutic strategies for muscular disorders is becoming possible thanks to new disease modeling strategies using iPSCs. Here, we describe arapid method to efficiently generate contractile myotubes from healthy or Duchenne Muscular Dystrophy (DMD) patient-derived iPSCs, based on the ectopic expression of MyoD and BAF60C. We show that homogeneous myogenic conversion of iPSCs is guaranteed by a BAF60C-dependent restriction of MyoD activity towards the skeletal muscle genes, while inhibiting alternative lineage programs. We took advantage of our unique cellular model to study muscular fibrosis associated with muscular pathogenesis. By using recombinant TGFβ1 we simulated the exacerbated fibrotic responsetypical of DMD muscles looking at the nuclear accumulation of phospho-SMAD (pSMAD3). We confirmed that DMD iPSC-derived myotubes display higher pSMAD2/3 signaling already in basal conditions and a higher responsiveness to TGFβ exposure, also when autocrine secretion of TGFβ was inhibited. The aberrant activation of the SMAD2/3 signaling was monitored in a high content screening and detected in both WT and DMD following "in-dish" contraction by electrical pacing, supporting the suitability of our method also for electrophysiological studies. We previously found that the histone lysine methyltransferase SETDB1 plays a crucial role during skeletal muscle differentiation by precluding precocious activation of muscle genes. Based on recent evidence on the association between SETDB1 and SMAD proteins, we investigated the involvement of the

SETDB1/TGFβ in the fibrotic response and found that in DMD myotubes the nuclear accumulation of SETDB1 was more persistent as well as the transcriptional activation of TGFβ downstream targets. Our results point to an unprecedented role of the epigenetic factor SETDB1 in the regulation of TGFβ response in myotubes and in the fine-tuning of the fibrotic response.

Program Abstract #34
Human iPS Cell-Derived Artificial Skeletal Muscles for Complex Disease Modeling of Muscular Dystrophies
Shilpita Sarcar[1], Luca Pinton[1,2], Sara Maffioletti[1], Heather Steele-Stallard[1,2], Moustafa Khedr[1], Daniel Moore[1], Alexander Henderson[1], Ingra Mannhardt[3], Louise Moyle[1], Giulia Ferrari[1], Dominic Wells[4], Francesco Muntoni[5], Rickie Patani[6], Thomas Eschenhagen[3], Peter Zammit[2], Francesco Saverio Tedesco[1,5]
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Skeletal muscle is the most abundant human tissue and its regenerative capacity is compromised in severe incurable diseases such as muscular dystrophies. Therefore, generating artificial human skeletal muscle is instrumental for investigating muscle pathophysiology and developing novel therapies. However, most bioengineering platforms are challenged by the limited expansion potential and differentiation ability of tissue-derived myogenic cells. To overcome these limitations, we have generated three-dimensional (3D) artificial skeletal muscle tissue from human embryonic and induced pluripotent stem (iPS) cells, including iPS cells from patients with Duchenne, Limb-Girdle and congenital muscular dystrophies. Skeletal myogenic differentiation of pluripotent cells was induced within hydrogels under tension to provide alignment. The resulting artificial muscle models recapitulated structural and functional characteristics of human skeletal muscle. Furthermore, fully human iPS cell-derived models containing key isogenic cellular constituents of normal skeletal muscle, including vascular endothelial cells, pericytes and motor neurons have been generated. This 3D model has also provided us with a platform to study pathological hallmarks of severe muscular dystrophies caused by mutations in the *LMNA* gene (known as muscle laminopathies), encoding the nuclear envelope protein LAMIN A/C, namely: *LMNA*-related congenital muscular dystrophy, Emery-Dreifuss muscular dystrophy (type 2 and 3) and limb-girdle muscular dystrophy 1B. Modeling in 3D constructs resulted in recapitulation of nuclear shape abnormalities (characteristic of laminopathies) with higher fidelity than using standard bi-dimensional cultures and identified nuclear length as a reproducible phenotypic readout. Finally, we will present current developments of this novel 3D platform for gene therapy and drug screening in muscle laminopathies and beyond. Funding: ERC, NIHR, BBSRC, MDUK, AFM-Telethon, EU FP7 PluriMes.

Program Abstract #35
96-well human skeletal muscle microtissue platform for disease modeling and drug testing
Penney Gilbert
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Traditional 2D skeletal muscle cell cultures do not support contractile apparatus maturation or lend themselves to measurement of contractile force. In contrast, human muscle cells derived in 3D culture mature robustly and can support electrical or chemically stimulated contractions. One benefit of these 3D in vitro models is their utility in phenotypic drug screens. However, current methods are limited in scalability, and measurement of contractile force is laborious, which prevents drug testing applications. Here we report development of a human muscle micro-tissue (hMMT) platform capable of generating tissues in bulk. The platform consists of a custom polydimethylsiloxane 96-well plate in which each well contains two micropost anchor points that act as artificial 'tendons' to support 3D hMMT maturation across the axis of uniaxial tension. hMMTs contract in response to chemical and electrical stimulations and produce measurable micropost deflections. We established the relationship between micropost deflection and contractile strength using a microsquisher and we developed a semi-automated MATLAB-

based tool to quantify force measurements from short video sequences. By integrating an optogenetic engineering approach, we implemented the platform for the study of contraction-mediated maturation and to the identification of myokines that elicit autocrine effects on microtissue maturation. Finally, we demonstrate the value of these tools, technologies, and methodologies for modeling and studying Duchenne Muscular Dystrophy in-a-dish. This platform provides a powerful pre-clinical system to assess the impact of candidate compounds on human skeletal muscle strength and health.

Program Abstract #36
In-vivo Transcriptomic Profiling of Systemic Aging using Encapsulated Skeletal Muscle Progenitors
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Sustained exposure to a young systemic environment rejuvenates aged tissues and promotes stem cell function. However, due to the intrinsic complexity of tissues it remains challenging to pinpoint direct effects of circulating factors on specific cell populations. Here we describe a method for the encapsulation of human skeletal muscle progenitors in highly diffusible polyethersulfone hollow fiber capsules that can be used to profile systemic aging independent of heterogeneous physical cellular interactions *in-vivo*.

Program Abstract #37
Engineering combinatorial biomimetic environments to control muscle stem and progenitor cell regulatory phenotypes and to enhance muscle cell transplantation therapies
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Skeletal muscle stem cells (MuSCs) are an adult stem cell population essential for muscle homeostasis and regeneration throughout adulthood. Clinical muscle cell therapies are limited by the rarity of MuSCs and the lack of bona fide self-renewal in myogenic progenitors. Thus, long-term adult MuSC expansion to a clinical-scale yield is a critical unmet need for cell-therapeutic approaches to treat acute and chronic muscle defects. To address this challenge, we use high-throughput microprinted arrays on tunable stiffness hydrogels for assessing combinatorial effects of mechanical and chemical signals on MuSC and myoblast phenotype. We examined combinatorial cytokine stimulation of murine myoblasts, which revealed time-dependent synergisms in phosphoprotein signaling dynamics. Partial least-squares regression modeling using a cue-signal-response paradigm accurately predicted phenotypic response outcomes. For example, immediate p38 pathway upregulation was anticorrelated with differentiation, but the correlation flipped if p38 was upregulated in extended culture. Using these insights, long-term MuSC culture environments were fabricated with hydrogels at varying rigidities conjugated with MuSC niche proteins to facilitate MuSC adhesion. FACS-isolated MuSCs were cultured on the gels for 5 weeks and stimulated with soluble growth factors and inflammatory cytokines transiently upregulated during the in vivo muscle repair process. Proliferation on laminin-coated 12 kPa hydrogels was enhanced by stimulation with a cocktail of bFGF, IL-1α, IL-13, TNFα, and IFNγ via activation of the JNK and p38 pathways. Combined cocktail stimulation and late-stage p38 pathway inhibition yielded >10^7-fold expansion and maintained MuSC transplantation potential for 5 weeks of culture. These findings suggest that large pools of MuSCs can be obtained through long-term self-renewal expansion in designed microenvironments while maintaining therapeutic potential and minimizing loss of phenotype.

Program Abstract #38
Efficient, non-viral in vivo gene editing of skeletal muscle
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UCLA, USA
The development of versatile genome-editing tools, such as CRISPR/Cas9, has raised hope and excitement about possible treatment of genetic muscle disorders such as Duchenne Muscular Dystrophy. Safe and efficient delivery of CRISPR/Cas9 into patient cells remains a great challenge. Therapies based on viral delivery are costly and result in long-term tissue expression of the CRISPR/Cas9 gene editing complex, which can increase unwanted off-target effects. We previously reported iTOP, a unique new method for the*in vitro*delivery of the recombinant CRISPR/Cas9 ribonucleoprotein (RNP) complex into cells (D'Astolfo 2015, *Cell*161:674-690). Here we demonstrate for the first time, the efficient, non-viral delivery of recombinant CRISPR/Cas9 RNP into skeletal muscle *in vivo*. We've characterized and quantified gene editing at single-fiber resolution and demonstrate that each fiber contains multiple, independent editing events. Single-cell mRNA sequencing revealed that different cell types in addition to the muscle fibers are targeted by *in vivo*iTOP delivery in skeletal muscle. iTOP delivery of CRISPR/Cas9 allows efficient and consistent gene editing in over 80% of the muscle fibers without prior tissue conditioning. Although iTOP relies on the tissue delivery recombinant Cas9 protein, we demonstrate conclusively that pre-existing immunity against Cas9 protein does not affect editing efficiency. Finally, we demonstrate that iTOP-mediated delivery of CRISPR/Cas9 protein can be applied in a wide variety of skeletal muscle types and allows robust and consistent restoration of Dystrophin expression in a murine model of Duchenne Muscular Dystrophy. Our data demonstrates that iTOP delivery of recombinant CRISPR/Cas9 provides a simple, safe and consistently efficient tool to manipulate the skeletal muscle tissue *in vivo*. This work was supported by the Princes Beatrix Spierfonds, Health Holland, the FSHD Foundation and Stichting Singelswim Utrecht.

Program Abstract #39
Recovery macrophages stimulate myogenic stem cell fusion during post-injury skeletal muscle regeneration
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During normal skeletal muscle regeneration, macrophages play important roles by delivering cues for sustaining coordinated myogenesis, controlling angiogenesis and regulating fibroblastic cell survival to ensure appropriate tissue repair. Particularly, recovery (anti-inflammatory) macrophages that are present during the second phase of muscle regeneration strongly sustain the last step of myogenesis including differentiation of myoblasts into myocytes and their fusion into multinucleated cells. Through a screening approach, we have identified a series of molecules that are secreted by anti-inflammatory macrophages and that play a direct role in myogenesis, among which RNAseT2. RNAseT2 did not impact myogenic cell differentiation, i.e. the kinetics of myogenin expression, but specifically stimulated myogenic cell fusion. Gain and loss of function in macrophages, tested in coculture experiments with myogenic stem cells, confirmed the specificity of its action on myogenic cell fusion in vitro. Gain of function experiments in vivo, using plasmid electroporation, validated its stimulating effect of fusion during skeletal muscle regeneration, assessed by an increased number of myonuclei in the regenerating myofibers. The molecular mechanisms of the mode of action of macrophage-derived RNAseT2 on myogenic cell fusion will be discussed. benedicte.chazaud@inserm.fr www.musclestem.com www.inmg.fr

Program Abstract #40
Roles for glycosylation in modulating ECM during neuromuscular development and homeostasis
Erin Bailey, Sarah Alrowaished, Elisabeth Kilroy, Emma Crooks, Daisy Drinkert, Chaya Karunasiri, Joseph Belanger, Andre Khalil, Joshua Kelley, Clarissa Henry
University of Maine, United States
Secondary dystroglycanopathies are muscular dystrophies that result from mutations in genes that participate in Dystroglycan glycosylation. Glycosylation of Dystroglycan is essential for muscle fibers to adhere to the muscle extracellular matrix (myomatrix). Although the myomatrix is disrupted in a number of secondary dystroglycanopathies, it is unknown whether improving the myomatrix is beneficial for these conditions. We previously determined that NAD+ supplementation is sufficient to improve muscle structure and the myomatrix in a zebrafish model of primary dystroglycanopathy. Here, we investigate how NAD+ affects neuromuscular phenotypes in zebrafish fukutin-related protein (*fkrp)* morphants

modeling FKRP-associated secondary dystroglycanopathy. We found that NAD+ supplementation prior to muscle development improved muscle structure, myotendinous junction structure, and muscle function in *fkrp* morphants. As movement also requires neuromuscular junction formation, we examined early neuromuscular junction development in *fkrp* morphants. The length of neuromuscular junctions was disrupted in *fkrp* morphants. NAD+ supplementation prior to neuromuscular junction development improved length. Ubiquitous overexpression of Fkrp rescued the *fkrp* morphant phenotype but muscle-specific overexpression only improved myotendinous junction structure. These data indicate that Fkrp plays an early and essential role in muscle, myotendinous junction, and neuromuscular junction development. Our data also indicate that FKRP-associated dystroglycanopathy does not exactly phenocopy DG-deficiency. NAD+ supplementation improves NMJ morphology in *fkrp* morphants but not *dag1* morphants. Finally, these data show that muscle-specific expression of Fkrp is insufficient to rescue muscle development and homeostasis. Funding: March of Dimes Award number #1-FY14-284 to C.A.H., NICHD R15HD088217 to C.A.H., and the University of Maine Chase Distinguished Research Assistantship to E.C.B.

Program Abstract #41
The extracellular matrix protein Tenascin-C is required to maintain the muscle stem cell pool and to promote regenerative potential
Mafalda Loreti, Alexandre Rosa-Campos, Alessandra Sacco
SBP Medical Discovery Institute, USA
Skeletal muscle regenerative capacity is progressively lost with aging and muscle-associated diseases due, in part, to the decline in number and function of muscle stem cells (MuSC). Several studies identified a critical role of the microenvironment in instructing MuSC behavior and temporally coordinating tissue maintenance and repair. However, the impact of these microenvironmental cues on MuSC function is still poorly understood. We have recently shown that Tenascin-C (TnC) promotes fetal MuSC expansion and enhances muscle tissue repair. This extracellular matrix (ECM) glycoprotein is mainly expressed during embryonic development and transiently upregulated during wound healing. TnC contributes to the interaction of cells with their microenvironment, regulating their adhesion, migration and differentiation. Here we show that genetic deletion of TnC results in perinatal reduction in MuSC numbers in skeletal muscle. During muscle repair, mice lacking TnC have smaller myofibers, suggesting that ablation of TnC leads to an impaired regenerative response. To uncover the molecular mechanism underlying this phenotype, we optimized a mass spectrometry approach to define the glycosylation sites of TnC and its binding partners in embryonic and adult muscle. We identified N1394 glycosylation site located in the FNIII-9 domain of TnC, which is conserved across vertebrates. We further identified a set of ECM binding partners of TnC, including Fibrillin-2 (Fbn2). Mutations in this ECM gene cause Beal syndrome, an inherited connective tissue disorder. Previous studies reported that Fbn2 sequesters BMP and TGF-ß in a latent state to maintain tissue homeostasis. During muscle repair, ablation of TnC induces an activation of BMP signaling, suggesting that TnC modulates this pathway. Ongoing experiments are focusing on defining the TnC-ECM interactome and understanding its impact on MuSC function during muscle development and repair.

Program Abstract #42
Therapeutic approaches to prevent FAP-directed heterotopic ossification in FOP
David Goldhamer
University of Connecticut, United States
Heterotopic ossification (HO), the formation of bone in skeletal muscles and associated soft tissues, is manifested in its most extreme form in the rare genetic disease, fibrodysplasia ossificans progressiva (FOP), which is caused by a gain-of-function mutation in the BMP receptor, ACVR1 [ACVR1(R206H)]. FOP patients suffer progressive and life-long severe disability as a cumulative consequence of broadly distributed HO, which interferes with skeletal muscle function, and results in joint ankylosis and other complications that dramatically decrease quality of life and life expectancy. We have shown that fibro/adipogenic progenitors (FAPs) are a major contributor to HO in an accurate genetic mouse model of FOP. Potential therapeutic approaches for FOP being tested in pre-clinical models and clinical trials target ligand-receptor interactions, receptor kinase activity and skeletogenic differentiation. Our experiences with these modalities and their effects on FAP behavior will be discussed. We will also

present data supporting the conclusion that the stoichiometric balance of wild-type to mutant ACVR1 receptors is a key modulator of disease severity. Specifically, both FAP-targeted and widespread over-expression of wild-type ACVR1 protected FOP mice from injury-induced HO and did not demonstrably affect embryonic or postnatal development. Collectively, these data suggest that ligand bioavailability rather than the cell specificity and absolute levels of ACVR1 expression are central modes of regulating ACVR1-dependent pathways. Possible mechanisms by which wild-type ACVR1 over-expression mitigates the pathological effects of ACVR1(R206H) will be discussed. This work was funded by NIH grant R01 AR072052.

Program Abstract #43
A tissue damage independent murine model to study muscle mass loss in Duchenne Muscular Dystrophy (DMD)
Nasim Sadat Kajabadi, Marcela Low, Chih Kai Chang, Fabio Rossi
University of British Columbia, Canada
Fibro/adipogenic progenitor (FAP)s are a subset of muscle resident mesenchymal progenitors that provide trophic support in regeneration but in chronic damage such as that caused by muscular dystrophies, differentiate to the modified extracellular matrix pathognomonic of fibrosis (Joe et al. 2011). In DMD, fibrosis is the metric that most strongly correlates with loss of muscle strength, but whether the two are causally linked is not clear. The study of the effects of fibrosis on tissue maintenance and function is limited by the lack of a model in which this condition can be induced without tissue damage (Wells 2018). We generated a new murine model in which fibrotic matrix can be induced by transgenic activation of β-catenin specifically in fibro/adipogenic progenitors (FAPs), the cellular effectors of fibrosis. Tamoxifen treatment of these animals leads to interstitial fibrosis as well as a dramatic decrease in myofiber size. Interestingly, β-catenin Knock out in FAPs rescued muscle atrophy in mdx mice. Analysis of RNAseq dataset of sorted FAPs in which β-catenin was activated, revealed upregulation of the inhba message coding Inhibin-βA, which upon homodimerization forms Activin receptor 2A ligands - a member of TGFβ family. It guided our hypotheses related to the mechanism underlying the loss of muscle mass after β-catenin activation in FAPs. To address that, we treated the animals with recombinant soluble human ActR2A-Fc fusion, an inhibitor of this signaling pathway. Changes in muscle weight showed that ActR2A-Fc treatment of mice with β-catenin activated FAPs, mitigates muscle wasting. In conclusion, β-catenin activation in FAPs exerts an interstitial fibrotic effect through ActivinA enhancement resulting in muscle atrophy which is reminiscent of pathological hallmark in DMD patients and suggests a therapeutic approach for patients suffering from muscle atrophy, specifically directed towards this molecule.

Program Abstract #44
Correction of Duchenne Muscular Dystrophy by Genome Editing
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Duchenne muscular dystrophy (DMD) is a severe, progressive muscle disease caused by mutations in the Dystrophin gene, which encodes a large intracellular protein that maintains integrity of muscle cell membranes. More than 4,000 DMD mutations have been identified in humans. The majority of mutations are deletions that cluster in hot spots, such that skipping of out-of-frame exons can potentially restore the reading frame of the Dystrophin protein. We have used CRISPR/Cas9 to generate new mouse models of DMD lacking the most prominently deleted Dystrophin exons in humans. To permanently correct DMD by skipping mutant dystrophin exons in postnatal muscle tissue in vivo, we have used adeno-associated virus-9 (AAV9) to deliver CRISPR/Cas9 gene editing components to dystrophic mice, a method we refer to as Myoediting. To enable the visualization of dystrophin gene editing in vivo, we have created mice harboring a luciferase reporter in-frame with the dystrophin genomic coding region. We have also optimized Myoediting of many types of DMD mutations in muscle cells derived from iPS cells generated from blood samples of DMD patients. In a path toward clinical translation of gene editing for DMD, we have recently restored dystrophin expression of dogs with DMD following systemic administration of gene editing components with AAV9. Opportunities and challenges in the path toward permanent correction of disease-causing mutations responsible for DMD and other monogenic disorders by genomic editing will be discussed.

Program Abstract #45

The humanization of rodent muscular dystrophy: are plasma lipids part of the problem?

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Cholesterol-rich membrane microdomains (CRMM) play a key role in the specific localization of signaling proteins and complexes that regulate cellular homeostasis. Proteomics screening of CRMM from cultured vascular endothelial cells have revealed the presence of many dystrophin glycoprotein complex (DGC)-associated proteins in these non-muscle cells including Dysferlin (LGMD2b) and Dystrophin (Duchenne MD), which suggested an unexpected link between blood vessels and skeletal muscle tissues. When notoriously mild models of MD were bred to ApoE-null mice, a common model of vascular disease and hyperlipidemia, we observed a drastic exacerbation of skeletal muscle wasting and fibro-fatty replacement, reaching near complete ambulation dysfunction. These data suggested that higher plasma lipoprotein levels can either be directly deleterious to MD muscles, or inhibit regeneration, two processes currently investigated in our laboratories using injury assays. We also noted that various MD animal models show abnormal plasma lipoprotein levels, highlighting defects in how these animals handle lipids. These data could explain how Duchenne MD medication losartan does not appear to prevent muscle wasting in LGMD2b due to differences in lipid handling. The relevance of these findings to the human condition will be discussed, as well as how CRMM could be at the center of another fragile 'health vs disease' equilibrium.

Program Abstract #46

Restoration of dystrophin at critical sites of expression following exon skipping

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Neuromuscular junction, myotendinous junction and muscle stem cells are central components of skeletal muscle and required for its excitation, force transmission and regeneration. Loss of dystrophin defects their respective function. We have generated two dystrophin reporter mouse lines, wild-type *Dmd*^{EGFP} and dystrophic *Dmd*$^{EGFP\text{-}mdx}$, in which dystrophin is fused to EGFP at its C-terminal end. This allowed, for the first time in mammals, direct visualization of dystrophin expression from the natural locus. Multiphoton microscopy in sedated *Dmd*^{EGFP}mice as well as live cell imaging of isolated muscle fibers revealed extremely strong dystrophin expression at the myotendinous and neuromuscular junction, whereas low dystrophin expression was revealed in costameric organization along the sarcolemma. Further, dystrophin was highly expressed in a subset of muscle satellite cells. Systemic treatment of *Dmd*$^{EGFP\text{-}mdx}$mice with tricycloDNA antisense oligonucleotides to skip mutated *Dmd*exon 23 restored high dystrophin expression at these critical sites as well the low costameric expression along the sarcolemma. Intriguingly, dystrophin restoration started at critical expression sites before being restored along the sarcolemma. We found that skeletal muscle reversed its dystrophic morphology towards wildtype histological appearance. Experiments are ongoing to determine whether restoration of dystrophin at critical sites is prerequisite for normalization of muscle function and recovery from dystrophic changes. We argue that dystrophin restoration at critical sites of expression is required for therapies to be curative. However, current standards of dystrophin quantification for preclinical and clinical research entirely lack respective information as Western blot and histology at midbelly muscle level fail to inform about dystrophin restoration at critical sites. Novel methods are required to improve imaging of restored dystrophin and to better predict its therapeutic benefit.

Program Abstract #47

CRISPR editing therapy for Duchenne muscular dystrophy in murine and canine models

Dongsheng Duan

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Duchenne muscular dystrophy (DMD) is the most common lethal childhood muscle wasting disease. It is caused by mutations that abolish the reading-frame of the dystrophin gene. CRISPR gene editing is a highly powerful technology that can restore the disrupted reading-frame at the DNA level. Adeno-

associated virus (AAV) is by far the most robust gene therapy vector for treating inherited neuromuscular diseases. Recent studies from several groups, including us, suggest that intramuscular or intravenous injection of tailored AAV CRISPR vectors can correct the mutated dystrophin gene and ameliorate muscle disease in murine DMD models. While these findings are encouraging, several important questions remain to be addressed such as the durability of the therapy, satellite cell editing, and translation to large mammals. Here we show that a single systemic co-delivery of an AAV9.Cas9 vector and an AAV9.gRNA vector to young adult mdx mice resulted in life-long correction of the mutated dystrophin gene, reduction of muscle pathology and improvement of skeletal muscle and cardiac function. Interestingly, we found that long-term therapy required co-delivery of more gRNA vectors. Using a muscle transplantation model, we further demonstrated that AAV9 CRISPR vectors efficiently transduced and edited Pax7 positive satellite cells. Finally, we tested AAV8 and AAV9 CRISPR vectors in three independent canine DMD models that harbor different mutations. Intramuscular injection of the AAV CRISPR vectors in adult dystrophic dogs successfully restored dystrophin expression in all three canine models. Our findings support further development of AAV CRISPR therapy for DMD. (Supported by NIH AR-69085, DoD MD150133, Hope for Javier, and Jackson Freel DMD Research Fund)

Program Abstract #48
Exploring mechanisms of FSHD with chronic or transient DUX4 expression in mice
Michael Kyba
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Facioscapulohumeral muscular dystrophy (FSHD) is caused by loss of repression of the normally silent *DUX4* gene, however the disease mechanism is enigmatic, not least because the DUX4 protein has proven notoriously difficult to detect in human muscle biopsies. We have developed the variable and reversible iDUX4pA-HSA mouse model and describe work investigating the effect of muscle fiber-specific long-term low level, or transient, DUX4 expression.

Program Abstract #49
Consequences of DUX4 Expression in vitro and in vivo.
Rebecca Resnick[1], Sean Shadle[1], Amy Campbell[1], Chao-Jen Wong[1], Leo Wang[2], Rabi Tawil[3], Silvère van der Maarel[4], Guo-Liang Chew[1], Robert Bradley[1], Stephen Tapscott[1]
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The mis-expression of the transcription factor DUX4 in skeletal muscle causes fascioscapulohumeral muscular dystrophy (FSHD). Normally expressed in the testes, most likely in the germline, and at the four-cell cleavage stage in human embryos, DUX4 activates a set of genes and repetitive elements that characterize the first wave of zygotic gene activation in the early embryo. When mis-expressed in skeletal muscle or other somatic cells, DUX4 induces a similar set of early developmental transcripts but also causes cell death, at least partly through a program of apoptosis. Several mechanisms have been identified for the DUX4-toxicity in somatic cells, including the activation of the double-stranded RNA response pathway. The mechanisms of activating this pathway in FSHD muscle cells, however, remains unknown. Nor is it known whether a similar induction of double-stranded RNAs occurs when DUX4 is normally expressed in development when cells survive the expression of DUX4. Our studies have identified components of the toxic pathways in DUX4 expressing muscle cells and their activity in normal development. In addition, we have recently identified DUX4 expression in a wide variety of cancers where the expression of DUX4 suppresses MHC Class I expression and promotes immune evasion. Together with ongoing studies analyzing RNA expression from MRI-informed biopsies of FSHD skeletal muscle, we are generating a deeper understanding of the normal role of DUX4 in development and the consequences of its mis-expression in skeletal muscle that leads to disease. NIH NINDS and NIAMS, Friends of FSH Research, Chris Carrino Foundation for FSHD funded this work.

Program Abstract #50
PAX7 and DUX4 in Facioscapulohumeral Muscular Dystrophy: a tale of two proteins and two cell fates
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Facioscapulohumeral muscular dystrophy (FSHD) is an incurable, inherited skeletal myopathy linked to overexpression of the transcription factor DUX4, which may drive FSHD pathology via both induction of target genes and inhibition of the function of the myogenic master regulator PAX7. Biomarkers for FSHD have classically focused on DUX4 target gene expression in skeletal muscle. However, DUX4 is notoriously difficult to detect in myogenic cells from FSHD patients. We demonstrated in meta-analysis across 9 FSHD transcriptomic datasets profiling muscle biopsies and single myocytes, that PAX7 target gene repression is a superior biomarker to DUX4 target gene expression. Moreover, PAX7 target gene repression correlates with active disease, independently to DUX4 target gene expression (Banerji and Zammit, 2019). Curiously, the efficacy of DUX4 target genes as an FSHD biomarker improves on muscles that have been identified as undergoing pathological inflammation by MRI and histology. Gene expression changes associated with the immune system have also been reported in FSHD muscle biopsies and DUX4 target genes are enriched for inflammatory processes, raising the question of whether DUX4 is expressed in the immune system. Performing RNA-seq on lymphoblastoid cell lines (LCLs) isolated from FSHD patient blood, we found more robust DUX4 and DUX4 target gene expression than FSHD patient-derived myoblasts and myotubes. Furthermore, we demonstrate that a set of genes elevated in FSHD LCLs is similarly elevated in FSHD muscle biopsies, and correlates with the level of DUX4 target gene activation, as well as histological inflammation. Crucially, these genes are unaltered in FSHD myoblasts and myotubes, implying a non-muscle source in biopsies. Our findings demonstrate an immune system source of DUX4 in FSHD, revealing novel molecular and cellular mechanisms driving FSHD pathology, amenable to therapeutic investigation. Banerji and Zammit 2019. ***Hum. Mol. Genet.*** **28**: 2224-2236.

Program Abstract #51
Modifiers for myopathy
Elizabeth McNally
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Many different single gene mutation account for muscular dystrophy. However, even in the presence of similar or even identical mutations, there is variable expressivity and penetrance. Using an intercross strategy in a mouse model of muscular dystrophy, we conducted genomewide mapping to discover genetic modifiers of muscular dystrophy. A quantitative trait locus mapping approach was used to first identify genetic regions that segregate with mild and severe forms of disease. Genetic regions were then interrogated using whole genome sequence of parental mouse strains as well as deep RNA sequencing to evaluate gene expression and splicing. Using this integrated approach, we identified multiple genetic modifiers of muscular dystrophy. The first modifier mapped encodes a latent TGF-beta binding protein. Differential binding to TGF-beta as well as the TGF-beta family member myostatin identified a mechanism that regulates both muscle growth as well as muscle fibrosis. Annexin A6 was identified as an independent modifier of muscular dystrophy. Annexin A6 is a protein highly expressed in muscle that mediates sarcolemmal repair. Using high resolution microscopy, we identified annexin A6's role in resealing disrupted membranes, including forming the repair cap at the site of muscle injury. Anxa6, which encodes annexin A6, is also a target of glucocorticoid steroids, which are used to treat muscular dystrophy. Together these data explain the variability due to primary gene defects and these modifier genes also highlight pathways to treat multiple forms of muscle disease. Supported by NIH, Parent Project Muscular Dystrophy and Department of Defense.

Program Abstract #52
Plasma cholesterol is a key regulator of muscle homeostasis and damage in Muscular Dystrophy
Zoe White[1,2], Marine Theret[1,3], Nadia Milad[1,2], Fabio Rossi[1,2,3], Pascal Bernatchez[1,2]
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Rationale: We have recently demonstrated that dysferlin-deficient, Limb-Girdle MD 2b (LGMD2b) mice exhibit prominent gait abnormalities, exacerbation of muscle wasting, and vast fibroadipogenic regions in MD-affected muscles when their non-high density lipoprotein (non-HDLc) cholesterol levels are

increased. This was achieved by Apolipoprotein E (ApoE) gene inactivation, a common model of hyperlipidemia, and a high-fat, 0.2% cholesterol-containing diet (HFD), which suggested that plasma lipids may be at the centre of the muscle wasting process in MD. Herein, wefurther investigated the role of cholesterol in dysferlin-deficient muscle wasting using dietary and in vivo models of experimental damage. **Results:** In severe Dysferlin/ApoE DKO mice, increasing dietary cholesterol to 2% drastically accelerated MD disease progression, resulting in profound ambulatory dysfunction and the euthanasia of 100% of mice at 4-5mo. of age, compared to 0% of those fed a standard HFD. Elevated total plasma cholesterol (up to 48mmol/L), highly enriched in bad' low-density lipoproteins, caused severe muscle wasting (49% reduction in size; $P<0.001$) and induced profound intramuscular fat and macrophage infiltration ($MAC2^+$) in triceps muscle groups. Heightened infiltration of Dysferlin/ApoE-deficient macrophages ($CD11b^+/F480^+$; 163%; $P<0.05$) was also observed 3 days after *in vivo* experimental muscle damage (micro-pin injury), but only in the presence of hypercholesterolemia. **Conclusion**: Our data suggest that that elevated levels of atherogenic non-HDL cholesterol can lead to accelerated muscle damage and heighten the sensitivity of infiltrating inflammatory macrophages to plasma cholesterol. Supported by CIHR, Jain Foundation, Rare Disease Foundation.

Program Abstract #53

Human muscle satellite cells regenerate muscle independent of PAX7

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Skeletal muscle harbors its own stem cell called satellite cell. Satellite cells ensure postnatal muscle generation and regeneration. Loss of muscle mass characterizes muscular dystrophies and is a leading cause of poor prognosis in diseases such as cancer and cardiomyopathies. Muscle wasting cannot be treated. Attempts to utilize the potential of muscle stem cells for therapeutic purposes have not been successful yet. The transcription factor PAX7 defines muscle stem cells and is of pivotal importance for maintaining and regenerating muscle postnatally. We recently described a method to obtain PAX7-positive oligoclonal cell colonies with high regenerative potential from human muscle biopsy specimens (Marg et al., 2014 J Clin Inv). We now identified PAX7-negative muscle cell populations that were also able to regenerate muscle after transplantation into immunocompromised mice. These included myogenic cell colonies from a unique patient with complete absence of PAX7 due to a homozygous *PAX7*c.86-1G>A mutation (PAX7-null). By employing single cell RNA sequencing we characterized PAX7-null cells and other PAX7-negative cell populations (PAX7-neg) further. We find that human PAX7-negative myogenic cell populations display a high level of heterogeneity, but also important common features such as high expression of *MYF5*. Myogenic PAX7-null cells are also characterized by CLEC14A, a marker so far exclusively known in endothelial cells. In conclusion, myogenicity and the capability to regenerate muscle exist independent of PAX7. Our study extends the understanding of human myogenic cells and may lead to novel criteria to select human muscle stem cell populations for therapeutic purposes.

Program Abstract #54

Nuclear Pore Complexes in the Regulation of Muscle Homeostasis

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Nuclear pore complexes (NPCs) are multiprotein channels that connect the nucleus with the cytoplasm. NPCs are built by the repetition of 32 different proteins known as nucleoporins or nups. Due to their essential role in regulating nucleocytoplasmic molecule traffic, NPCs have been historically considered structures of ubiquitous composition. But it has recently become evident that the expression of nucleoporins varies significantly among tissues and that NCPs can be specialized to play specific cellular functions. Nup210 was the first described cell type-specific nucleoporin. We previously identified that this nuclear pore protein is induced during myoblast differentiation and is required for myotube formation and survival. Moreover, we determined that in Zebrafish Nup210 is plays a critical role in muscle growth, myofiber maturation and the survival of differentiated muscle cells. But whether Nup210 function in the physiology of skeletal muscle is conserved in mammals was unknown. Our recent work using a Nup210 mouse knockout line identified that animals lacking this nucleoporin shown a progressive deterioration of muscle structure and function. We also discovered that Nup210 knockouts exhibit histological and physiological features of inflammatory myopathies. Our findings support the idea that nuclear pores are

important regulators of muscle homeostasis and establish Nup210 as a key contributor to muscle maintenance in mammals. This work was supported by the Pew Biomedical Science Scholar Award and the National Institutes of Health (Awards #RO1AR065083 and #RO1AR065083-S1).

Program Abstract #55
Competition of mitochondrial genomes within mouse skeletal muscle fiber
Ekaterina Korotkevich, Eric Chow, Saul Villeda, Pat O'Farrell
UCSF, USA
Cells and organisms depend on flawless performance of mitochondria, which in turn relies on the integrity of the mitochondrial genome. A rich literature shows progressive accumulation of mtDNA mutations with age and implicates this accumulation in numerous age-related conditions such as sarcopenia and Parkinson's disease. Despite its importance, the factors underlying accumulation of mtDNA mutations remain obscure, and no experimental system directly connects aging symptoms to mutation accumulation. Here, we propose that mtDNAs compete with each other inside a cell and that selective forces guide the outcome. mtDNA with mutation conferring a replicative advantage will be positively selected and eventually take over the cell even if this mtDNA variant compromises mitochondrial function. Such over-replicating genomes are likely to arise frequently in skeletal muscle cells, that contain millions of mtDNAs. Thus, first, we set to characterize the spectrum of mtDNA mutations accumulating with age by deep sequencing of mtDNA from single mouse muscle fibers and fragments of various tissues. We identified 198 unique single point mutations across all examined samples. Seven of these mutations were detected in at least two mice and their abundance increased with age. Remarkably, 6 of these mutations localized in the D-loop, a control region where mtDNA replication is initiated, suggesting involvement of mtDNA replication in their accumulation. Notably, we also identified a deleterious mutation (12282G>A - premature stop codon in ND5 gene) accumulating with age in kidney, indicating presence of destructive selection. In the future we will test the competitiveness of mtDNAs possessing identified mutations and effects of their accumulation by transplantation into naïve muscle. Ultimately, the results of this project will bring new insights into mechanisms underlying mutant mtDNA expansion in mammalian somatic tissues and guide efforts to maintain mtDNA integrity. Funded by LLHF.

Program Abstract #56
Telomere length regulation of muscle stem cells in chronic injuries
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In chronic injuries such as Duchenne Muscular Dystrophy (DMD), the repeated cycles of muscle damage and repair lead to stem cell dysfunction. We and others previously showed that the muscle stem cell (MuSC) pool becomes less efficient at repairing damage in dystrophic mouse models. While progress has been made over the last decade with respect to potential treatments for DMD, current strategies are focused on treatment of skeletal muscle and do not take muscle stem cells into consideration. We recently demonstrated that telomere shortening is a district feature of dystrophic MuSCs in both mice and DMD patients already at a very young age. We have generated unique mouse tools that allow us to study stem cells within their native tissue environment of live mice and have determined the cellular consequence of telomere shortening in MuSCs. Furthermore, we discovered a previously unknown crosstalk between NF-kappaB and telomeres and determined the function of a telomeric protein in the progression of muscular dystrophy. These findings expand the fundamental knowledge of stem cell biology in diseased muscles. Understanding the molecular link between stem cell functional exhaustion and telomere shortening in DMD will significantly impact the conceptual view of DMD skeletal muscle pathology, providing fresh therapeutic perspectives on disease progression and will likely inform similar mechanisms in musculoskeletal applications with stem cell dysfunction, such as chronic or repeated muscle injuries in aging.

Program Abstract #57
Histone modifications and potential for differentiation therapy of rhabdomyosarcomas
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Rhabdomyosarcomas are a type of cancer known as sarcoma that are thought to be derived from mesenchymal stems cells or myogenic precursors. They can resemble developing skeletal muscle but appear blocked in their ability to terminally differentiate into skeletal muscle tissue. Histone modifications are tightly regulated during normal myogenic differentiation and play a key role in controlling the expression levels of genes involved in skeletal muscle development. Histone modifying enzymes such as the histone demethylase KDM1A, as part of the NuRD complex, and the histone methyl transferases KMT1A, G9a and EZH2, the latter as a component of the PRC2 (polycomb repressive complex 2), have been shown to impact on the growth and development of rhabdomyosarcomas. Evidence for these will be reviewed. EZH2/PRC2 is responsible for maintaining the transcriptionally repressive H3K27me3 mark and we have shown that silencing EZH2 causes non-terminal differentiation of rhabdomyosarcoma cells. Retinoic-based treatments can have similar effects but the combination of EZH2 inhibition with retinoic-based treatment results in more severe growth inhibition and greater myogenic differentiation than either treatment alone. A model that we are testing to explain these effects will be discussed. In-line with differentiation therapies for leukaemia and reducing risk of relapse in neuroblastoma, differentiation of rhabdomyosarcoma cells may be therapeutically beneficial to improve outcomes, especially in patients at high-risk of relapse.

Program Abstract #58
Epigenetic reprogramming in rhabdomyosarcoma
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Rhabdomyosarcoma, the most common paediatric soft-tissue tumours, are thought to arise from myogenic precursors unable to complete the differentiation program. Amongst the different subtypes, alveolar rhabdomyosarcomas (ARMSs) present the poorest prognosis and are characterized for the presence of PAX3/7-FOXO1 fusion products. As it occurs with most paediatric tumours, the mutation burden of ARMS is extremely low, suggesting that non-genetic mechanisms cooperate with the fusion protein to drive tumorigenesis. Therefore, much interest has been put in the past years in unveiling the epigenetic alterations driving rhabdomyosarcomagenesis. However, if and how the epigenome of tumour cells can be modulated by external interventions still needs to be addressed. Here we used a “multi-omics” approach to demonstrate that enforced activation of the p38α MAPK pathway induces terminal differentiation of ARMS via chromatin signalling. Mechanistically, we provide evidence of a functional interplay between PAX3-FOXO1, EZH2 and the DNA methylome, that converges at the Ras, Rap1 and PI3K/Akt1 pathways upon p38α activation. The described interplay identifies these oncogenic pathways as key hotspots for epi-mutations in ARMS, in the absence of genetic mutations. Interestingly, several components of these pathways are commonly mutated in embryonal rhabdomyosarcoma (ERMS) highlighting their importance in supporting tumour growth in most, if not all, rhabdomyosarcoma. The functional interplay described here provides mechanistic insights into how signalling cascades can be exploited to remodel the epigenome of tumour cells and allowed us to identify new targets for therapeutic interventions. *Funding:* Italian Ministry of Health (GR-2011-02349383), the Italian Association for Cancer Research (MFAG 14799), the Worldwide Cancer Research (12-0168) and the Euronanomed2 Joint Call (ER-2016-2360733).

Program Abstract #59
Loss of muscle stem cells quiescence in Suv4-20h1 mutants is associated with genomic instability and rhabdomyosarcoma formation
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Dynamic regulation of adult stem cell activity by epigenetic mechanisms is crucial to maintain tissue homeostasis. Adult skeletal muscle stem cells (MuSCs) are normally maintained in a quiescent state but get activated during skeletal muscle growth or after damage of muscle fibers. Loss of heterochromatin formation characteristic for quiescent MuSC evokes aberrant activation and proliferation of MuSCs, which compromises tissue regeneration and might also increase the risk of tumor development. Previously, we described that the H4K20 dimethyltransferase Suv4-20h1, which uses H4K20m1 as substrate, controls

quiescence of MuSC by promoting formation of facultative heterochromatin (fHC). We now report that loss of heterochromatin in MuSC-specific Suv4-20h1mutants is also associated with massively enhanced genomic instability and aberrant mitosis. Suv4-20h1 mutant MuSC rapidly form rhabdomyosarcomas in p53-deficient muscles while absence of p53 alone is not sufficient for tumor formation in skeletal muscles. Mechanistically, depletion of Suv4-20h1 results in strong accumulation of H4K201me1 over gene bodies, causing increased transcriptional elongation. Increased transcriptional elongation together with precocious DNA replication due to loss of nuclear periphery heterochromatin results in head-on transcription-replication collisions and formation of R-loops. R-loops are three-stranded nucleic acid structures, composed of DNA:RNA hybrids and associated non-template single-stranded DNA, which block replication forks to induce fork collapse and subsequent double-strand DNA breaks. Accordingly, we detected a massive increase of double-strand DNA breaks, micronuclei formation, multipolar mitotic figures and aneuploidy in Suv4-20h1 mutant MuSC, which explains the rapid formation of rhabdomyosarcomas in Suv4-20h1 mutant mice.

Program Abstract #60
Satellite cell contributions to pediatric skeletal muscle growth and cancer survivorship
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Advances in medicine have successfully raised the 5-year survival rate of children diagnosed with malignant cancer to nearly 80%; however, prior to the age of 40, the majority of these individuals suffer from age-related physical limitations normally associated with the elderly population. Sarcopenia is one of the age-associated phenotypes that is observed prematurely in childhood cancer survivors. We have found the critical importance of skeletal muscle stem cell (satellite cell) contribution to prepubertal skeletal muscle growth. These data suggest that childhood skeletal muscle growth is susceptible to insults and stressors that can jeopardize the contribution of juvenile satellite cells and derived myogenic progenitors. Cancer patients routinely undergo multiple modes of treatment including surgery, chemotherapy, or radiation; however, radiation makes up approximately 50% of cancer treatment. Although irradiation is a mainstay therapy of most juvenile cancer treatments, it is also one that acts by killing actively dividing cells through direct or indirect DNA damage via reactive oxygen species, which can be detrimental to the growth and maintenance of various tissues including skeletal muscle. Here we have developed a preclinical model for radiation-induced sarcopenia in the context of rhabdomyosarcoma (RMS); the most common pediatric soft tissue sarcoma. With this model we find that a clinically relevant fractionated x-ray radiation regimen using a Small Animal Radiation Research Platform (SARRP) can mitigate established RMS tumors at a rate of approximately 80%. Despite tumor mitigation, we find atrophy, reduction of vasculature, force generation deficits, and loss of satellite cell contribution and myonuclei in radiation-treated skeletal muscles. This was associated with persistent upregulation of inflammatory signaling pathways post-RMS radiation therapy that could impede remaining satellite cell contribution to muscle growth, maintenance, and regeneration.

Program Abstract #61
USP7 inhibition results in down-regulation of muscle-specific gene expression and differentiation impairment.
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Hierarchical expression of the myogenic regulatory family of transcription factors (MRFs) is key for muscle formation during development and tissue regeneration in adult stages. MRFs drive lineage commitment and terminal differentiation, and their function is highly regulated at the post-translational level, specially by ubiquitination via the ubiquitin-proteasome system. On the other hand, subunits of SWIF/SNF chromatin remodeling complex interacts directly with MyoD and Myogenin, initiating differentiation and muscle-specific gene expression. Importantly, MyoD and Myogenin interact with different subunits of this complex throughout the myogenic progression. Preliminary results from our laboratory support the idea that Ubiquitin-specific-protease (USP7), take part in gene expression regulation, likely by interacting with the MRF Myogenin. In other cells types, it is known that USP7 helps to maintain the conformation of transcriptional regulatory complexes and their interaction with SWIF/SNF complex. Our interest is to determine if USP7 functions in muscle-specific gene expression by regulating MRF and SWIF/SNF

interactions. Initial studies show that USP7 inhibition leads to differential changes in total mRNA expression in C2C12 cells maintained under proliferating and differentiating conditions. As a result, inhibition of USP7 results in down-regulation of muscle-specific gene expression in differentiating C2C12. These observations correlate with an overall differentiation impairment (e.g., myotube formation) upon USP7 loss of function. These results advise that USP7 activity is required to regulate muscle gene expression at specific stages of the myogenic progression. Alternatively, we suggest USP7 not only interact directly with Myogenin but also may be regulating chromatin remodeling or transcriptional complexes during early muscle differentiation. Acknowledgments: Fondecyt 1170975 & VRI grant.

Program Abstract #62

Bromodomain function of mammalian SWI/SNF (mSWI/SNF) chromatin remodeling enzymes is crucial for skeletal muscle differentiation.

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Muscle differentiation is a very tightly coordinated process involving changes in the epigenetic landscape of the precursor cells as they proceed towards a myogenic phenotype. mSWI/SNF protein complexes have been previously shown to be key regulators for initiation of the muscle differentiation program, where they coordinate with muscle-specific transcription factors. BRG1 and BRM are mutually exclusive ATPase motors of the mSWI/SNF complexes. Our previous studies have shown that amino acid residues flanking the Brg1 bromodomain need to be dephosphorylated for determining the myogenic fate. These residues are not conserved in the Brm ATPase. Consequently, we sought to examine the role of mSWI/SNF bromodomains in this process. Our results show that the bromodomain inhibitor PFI-3, which is specific for the bromodomains of three mSWI/SNF proteins (BRG1, BRM and BAF180), causes a loss of the muscle differentiation phenotype. Treated cells show decreased expression of myogenic markers and fusion related genes. Our RNA-sequencing studies identified an altered gene expression signature of cell-cycle and muscle-related genes in the presence of PFI-3. Comparison of our dataset with published Brg1/Brm knockdown datasets from Lorenzo Puri's group showed that expression of about one-tenth of total genes regulated by mSWI/SNF complexes depends on their bromodomain function. ChIP analysis of myogenic promoters revealed decreased occupancy of the mSWI/SNF bromodomain proteins on myogenic promoters in the presence of PFI-3. The studies, therefore, indicate that the bromodomains of the mSWI/SNF proteins play a role in myoblast cell cycle exit and differentiation by assisting recruitment of the mSWI/SNF complexes to specific loci, thereby contributing to myogenic differentiation. Funding Source: NIH grant GM56244 to ANI

Program Abstract #63

Functional properties of a novel mechanosensitive Ca2+-permeable channel in mouse derived satellite cells

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Skeletal muscle mass and function are mediated by mechanical stress such as stretch and loading. For instance, functional overloading stimulates the regrowth of matured skeletal muscle. Conversely, skeletal muscle atrophy is induced by unloading. Various mechanosensitiveCa^{2+}-permeable channels have been proposed as mechanosensors for skeletal muscle cells. However, the molecular mechanisms involved in the sensing of mechanical stress are not fully elucidated. In the present study, we investigated roles of a novel mechanosensitive Ca^{2+}-permeable channel in the importance of mechanosensation and myogenesis. Functional properties of a mechanosensitive Ca^{2+}-permeable channel of muscle satellite cells isolated from mouse skeletal muscles were evaluated by stretch, siRNA knockdown, antagonist or agonist-induced activation. Effects of modified mechanosensitive Ca^{2+}-permeable channels on myogenic progression in mouse satellite cells were also investigated by RT-qPCR and general immunolabelling techniques. The mechanosensitive Ca^{2+}-permeable channel in question was upregulated by myogenic differentiation in primary derived myoblasts. Myotube formation and growth were suppressed by inactivation of this mechanosensitive Ca^{2+}-permeable channel. Our evidence indicates that this novel

mechanosensitive Ca^{2+}-permeable channel plays an essential role in myogenic differentiation. Further, this Ca^{2+}-permeable channel functions as mechanosensor for mechanical stress-associated regulation of skeletal muscle mass and function. This study was supported, in part, by KAKENHI (Grant Numbers JP16K13022, JP17K01762, JP18H03160), the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan, and Graduate School of Health Sciences, Toyohashi SOZO University.

Program Abstract #64

Klotho influences myogenesis during postnatal growth and during muscle regeneration.

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Although the protein Klotho (KL) has been primarily studied in the context of aging, we found that KL expression is most dynamically regulated in muscle during the first weeks of postnatal growth. Our findings support a significant role for KL in regulating myogenesis in developing and regenerative muscles. First, the secreted form of KL is most-highly expressed in muscle cells *in vitro* when they are proliferative myoblasts, declining when differentiation occurs. In addition, treatment of myoblasts *in vitro* with KL increased proliferation and reduced the expression of transcription factors (MyoD; myogenin) and Wnt-signaling molecules that drive muscle differentiation. *In vivo* findings showed that expression of a KL transgene increased Pax7+ satellite cells in muscles of young mice (2- and 4-weeks of age) and reduced fiber cross-sectional area (CSA) at 2-weeks. At 12-weeks, fiber CSA was increased in transgenic mice, showing that KL promotes fiber growth in young adult mice. However, *in vitro* observations indicate that the KL-induced increases in growth require the presence of the KL co-factors, heparin and FGF23. KL plays similar regulatory roles in regenerating muscle. Following acute injury, increases in KL expression coincided with increased Pax7+ cells at 7-days post-injury. In addition, increasing KL levels in regenerating muscles by KL transgene expression further amplified the numbers of Pax7+ cells at 7-days and 21-days post-injury while accelerating fiber growth between 7- and 21-days post-injury. The transgene also reduced the proportion of Pax7+ cells expressing activated β-catenin at early stages of regeneration, showing inhibition of Wnt signaling. Collectively, these findings indicate that there is a developmental window in which satellite cells numbers can be significantly affected by changes in KL expression, which influences subsequent muscle fiber growth. (Supported by NIH grants T32GM065823, F31AR071782, F32AR065845, RO1AR066036 and RO1AR062579).

Program Abstract #65

Investigating the role of mechanical tension during sarcomere self-organisation in human iPSC-derived muscle fibers

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How does the actomyosin cytoskeleton break symmetry and self-organise into higher order structures in living cells? In the striking case of myofibrillogenesis during skeletal muscle development, actomyosin filaments self-organise simultaneously into chains of hundreds of micrometer-long sarcomeres in millimeter-long myofibers. How are the correct number of sarcomeres assembled to bridge the length of the muscle fiber? Our previous work in *Drosophila* indirect flight muscle has led us to hypothesize that tension works as a mechanical compass to coordinate sarcomere self-organisation simultaneously across the entire length of the myofibril. In this study, we aim to test whether the tension-driven myofibril self-organisation mechanism is conserved during myofibrillogenesis in human iPSC-derived muscle fibers generated *in vitro*. We will initially characterize the dynamics of sarcomeric components and muscle attachments in human iPSC-derived myofibers at key developmental time points. Further, we aim to quantify and manipulate tension via laser nanosurgery in differentiating human muscle fibers *in vitro*. The expected results should enable us to incorporate mechanical tension into a previously developed mathematical model purely considering molecular interactions between actomyosin filaments. Successful

completion of our research aims will uncover how tension instructs actomyosin interactions during sarcomere self-organisation. Our study will also provide insights into the role of mechanical stimulation on muscle regeneration and maintenance in an aging population.

Program Abstract #66
mRNA transport and stability interplay to localize mRNA in skeletal muscle
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Skeletal muscle is composed of multinucleated myofibers with nuclei dispersed along the cell periphery. Although in several muscular disorders these nuclei are misplaced, the relevance of muscle nuclear positioning in myofiber function remains unclear. The nuclear domain hypothesis suggests that mRNAs remain near the respective transcribing nucleus and therefore each nucleus influences a specific region in the myofiber. Using single-molecule FISH on *in vitro* differentiated myofibers and muscle sections, we identified several mRNAs that cluster around the nucleus of origin. Blocking transcription was sufficient to disrupt mRNA clustering, indicating that a balance between stability and transcription rates is the main mechanism for this perinuclear accumulation. Remarkably, mRNAs coding for muscle giant proteins – the giant mRNAs – are unexpectedly spread along the cell in comparison with non-giant mRNAs. This distribution is independent of the topology of the encoded protein (cytoplasmic vs transmembrane) and function (sarcomere vs triads). Giant mRNA clustering was only observed at the cell tip, where microtubule plus-ends are also enriched. In agreement, we found that giant mRNAs interact with Fragile X mental retardation proteins. These are RNA-binding proteins, previously associated with kinesin-mediated transport in neurons. We propose that in skeletal muscle, at least two mRNA localization mechanisms are in place: mRNA stability and transcription rates determine the localization of non-giant mRNAs in the perinuclear region and a microtubule-dependent transport specific for the distribution of giant mRNAs.

Program Abstract #67
Phosphorylation of MLC2 by PKCδ is required for flight muscle maturation in *Drosophila*
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PKC is a family of kinase enzymes, which activate downstream proteins by phosphorylation of serine and threonine residues of the targeted protein. PKCδ is a novel PKC isoform which is activated by diacylglycerol (DAG). *Pkcδ* is located on the X chromosome of the *Drosophila* genome. Myosin is an essential motor protein, comprised of two isomers that coiled around each other. Each isomer is made of three fragments: one Myosin Heavy Chain (MHC), and two Myosin Light Chains (MLC), which are categorized into two groups: 1- Essential or alkali MLC (MLC-1) and 2- Regulatory MLC (MLC-2). It has been shown previously that MLC2 extension and phosphorylation is essential for proper positioning of myosin heads and interacting with actin filaments, and we show that this phosphorylation occurs as adult flies emerge from the pupal case. We hypothesized that Pkcδ phosphorylates several Ser/Thr residues of MLC2. *Pkcδ* mutant flies are completely flightless, that can be rescued by duplication of the wild-type *Pkcδ* gene. We have shown that MLC2 protein is not phosphorylated in *Pkcδ* mutant flies by western blot analysis, but it is phosphorylated in duplicated flies with *Pkcδ* mutant background. Mass spectrometery results also indicated that threonine 38 of MLC2 is phosphorylated in wild type flies but not *Pkcδ* mutants. We will perform kinase assays on skinned myofibrils and purified myosin of *Pkcδ* mutant flies using PKCδ recombinant enzyme to determine if Pkcδ directly phosphorylates MLC2 protein. We will also determine the role of DAG production in the activation of PKCδ and thereby in the maturation of the flight muscles.

Program Abstract #68
HSP70-specific nuclear transporter Hikeshi in myogenic differentiation
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Heat shock protein 70 (HSP70) plays as a molecular chaperone that has essential roles in protein folding,

degradation, and transport in eukaryotic cells including skeletal muscle cells. Intracellular expression of HSP70 is upregulated by various cellular stresses such as heat stress. Nuclear accumulation of HSP70 in HeLa cells is observed in response to heat stress. It has been proposed that Hikeshi plays as a specific nuclear import carrier protein for HSP70 under stressful environment. Recently, we have demonstrated that HSP70 is translocated from cytoplasm to the nuclei in response to heat stress and gravitational loading in mouse skeletal muscle. However, the detail for physiological role of Hikeshi in skeletal muscle cells remains unclear. The purpose of this study was to investigate the role of Hikeshi in myogenic differentiation in cultured skeletal muscle cells. Mouse myoblasts-derived cell line C2C12 was used in this study. The protein and mRNA expression level of Hikeshi was evaluated. The effects of Hikeshi knockdown, which was induced by RNA interference using siRNA, on differentiation of C2C12 cells were investigated. Both mRNA and protein expression of Hikeshi was observed in both C2C12 myoblasts and myotubes. Although mRNA expression level of Hikeshi was stable during the differentiation, the protein expression level of Hikeshi was upregulated by the differentiation. Myotube growth was partially suppressed by the knockdown of Hikeshi. Total muscle protein content was significantly decreased by Hikeshi knockdown. Hikeshi may play a role in myogenic differentiation. This study was supported, in part, by KAKENHI (Grant Numbers JP16K13022, JP17K01762, JP18H03160), the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan, and Graduate School of Health Sciences, Toyohashi SOZO University.

Program Abstract #69

The Core Enhancer and Distal Regulatory Region are not necessary for MyoD expression during embryonic myogenesis

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Proper progression of myogenesis, during both development and regeneration, relies on appropriate expression of the myogenic regulatory factors: MYF5, MYOD, MYOG, and MRF4. Two enhancers have been shown to regulate *MyoD*, the Core Enhancer (CE) and the Distal Regulatory Region (DRR), which are located 20kb and 5kb upstream of the *MyoD* transcription start site, respectively. Previously, we demonstrated that targeted deletion of either the CE or DRR results in only modest disruption of embryonic *MyoD* expression, suggesting either redundancy between CE and DRR function or existence of additional, undiscovered *MyoD* regulatory elements. To assess functional overlap between the CE and DRR, both enhancers were knocked out by sequential gene targeting. Notably, deletion of both elements had only modest effects on *MyoD* expression, with maintenance of muscle-specific expression, as assessed by *in situ* hybridization. Deletion of a 20 kb sequence that includes both enhancers along with the 15 kb sequence between them had no additional effect on *MyoD* expression. Quantitative analysis using droplet digital PCR identified that levels of *MyoD* expression exhibited a dosage dependent effect between wild-type, heterozygous, and homozygous enhancer knockout embryos. This same trend was observed both in the developing trunk and limb bud, persisting throughout embryonic and fetal myogenesis. Together, these data suggest that additional undiscovered enhancer elements may regulate the timing, muscle specificity, and levels of *MyoD* expression during embryonic development. This project was funded, in part, by a grant from the NASA CT Space Grant Consortium to C.J.J.

Program Abstract #70

Single-cell analysis of mouse embryonic and fetal limb muscle populations

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Limb development is a complex process involving dynamic changes in individual cells' gene expression and signaling between emerging cell types. We used single-cell RNA sequencing and Cre-dependent lineage tracing at embryonic days (E) 11.5 and 17.5 to identify cell populations in mouse embryonic and fetal limbs, identify signals between populations, and dissect sub-populations of the myogenic lineage. At E11.5 we identified hematopoietic, endothelial, myogenic, and chondrogenic lineages as definitive populations, and a group of closely related clusters that highly express proliferative and mesenchymal markers. At E17.5, more populations became identifiable. These include skeletal myoblasts, smooth muscle, endothelial cells, keratinocytes, tenocytes, chondrocytes, and Schwann cell precursors. Within the myogenic cluster at E11.5, we identified four subpopulations, with one population exhibiting the

highest expression level of proliferative genes and genes known to inhibit muscle differentiation, such as *Top2a*, *Cdk1*, *Prmt3*, and *Sox9*. Additionally, one population had the lowest *Pax3* expression and highest *Myog* expression. At E17.5 the myogenic cluster was a higher percentage of the total observed cells and could be sub-clustered into five populations, including one population high in proliferative gene expression and one *Myog*-high population. Comparing the two timepoints, the *Myog*-high populations were more similar than all other populations. Genes high in the embryonic proliferative population included *Prdx6* and *Id2*, while the E17.5 proliferative cells strongly expressed *Dlk1*, *Mgp*, and *Sparc*, although both populations expressed proliferative genes such as *Ccnb1* and *Cdc20*. Implications for regulation of lineage determination and differentiation will be discussed. This project was funded by a grant from CT Space Grant Consortium to A.J.P.

Program Abstract #71
MyoD induced enhancer RNA interacts with hnRNPL protein via CAAA motif to activate target gene transcription during myogenic differentiation
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Emerging evidence supports active roles of enhancer RNAs (eRNAs) in regulating target gene expression but our understanding of the underlying mechanisms remains incomplete. Here, we study eRNA regulation and function using skeletal myoblast differentiation as a paradigm. We provide a panoramic view of enhancer transcription and dynamics during myogenic differentiation and first categorization of eRNAs by integrating GRO-seq and RNA-seq data. We demonstrate the essential role of master transcription factor MyoD in activating eRNA production. Subsequent in depth dissection of super enhancer (se) generated seRNA-1 and -2 uncovered that seRNAs can promote myogenic differentiation in vitro and in vivo. Mechanistically, we found these seRNAs control the transcription of target genes by specifically binding to heterogeneous nuclear ribonucleoprotein L (hnRNPL) and modulate hnRNPL dosage on the target promoter. A CAAA tract on seRNA-1 was further identified to be essential in mediating seRNA-1/hnRNPL binding and function. Disruption of seRNA-hnRNPL interaction attenuates Pol II and H3K36me3 deposition at the target genes, in coincidence with the reduction of their transcription. Furthermore, analyses of hnRNPL binding transcriptome-wide reveal its association with eRNAs is a general phenomenon in multiple cells. Collectively, we propose that eRNA-hnRNPL interaction represents a novel mechanism contributing to target mRNA activation.

Program Abstract #73
FGF signals direct myotube guidance by regulating Rho/Rac activity
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Nascent myotubes undergo a dramatic morphological transformation during myogenesis in which the myotubes elongate over several cell diameters and choose the correct muscle attachment sites. Although this process of myotube guidance is essential to pattern the musculoskeletal system, the mechanisms that control myotube guidance remain poorly understood. Using transcriptomics, we found that components of the Fibroblast Growth Factor (FGF) signaling pathway were enriched in nascent myotubes in *Drosophila* embryos. Null mutations in the FGF receptor *heartless* (*htl*), or its ligands, caused significant myotube guidance defects. Mechanistically, paracrine FGF signals to Htl in the mesoderm regulate the activity of Rho/Rac GTPases in nascent myotubes to effect changes in the actin cytoskeleton. FGF signals are thus essential regulators of myotube guidance that act through cytoskeletal regulatory proteins to pattern the musculoskeletal system. This work was supported by NIH R01AR070299, the WashU Musculoskeletal Research Center (NIH P30 AR074992), and the Boettcher Foundation.

Program Abstract #74
Expression of helix-loop-helix transcription factor Ascl4 induces myogenic program in embryonic stem cells
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The basic helix-loop-helix (bHLH) transcription factors play central roles in developmental processes including cell fate specification such as MyoD family for myogenesis and Achaete-scute complex-like 1 (Ascl1) for neurogenesis. Here, we found that all Ascl family (Ascl1-5) has the ability to induce myogenic program when overexpressed in embryonic stem cells (ESCs). Among them, we noticed that Ascl4 expression is detected in dermomyotome, a place for myogenic progenitor cells during mouse embryogenesis. In ESCs, overexpression of Ascl4 efficiently induces MyoD/Pax7-positive myogenic cells followed by myosin heavy chain-positive terminally differentiated myocytes when Ascl4 expression was withdrawn. Integrative analysis of RNA-seq and ChIP-seq data revealed that Ascl4 is able to induce MyoD expression through the binding of E-boxes (CAGCTG) in a novel MyoD enhancer region named embryonic enhancer region (EER) located at 60 kb upstream of its start site. In transgenic mice, overexpression of Ascl4 is able to expand myogenic region during mouse embryogenesis. Together, these findings imply that Ascl4 may mediate activation of MyoD as a key regulator of somitic myogenesis.

Program Abstract #75
The MuSK-BMP pathway is necessary for maintaining a sub-population of Type IIb muscle fibers
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Bone morphogenetic proteins (BMPs) play an important role in regulating muscle homeostasis. Recently our laboratory has shown that muscle-specific kinase (MuSK), a receptor tyrosine kinase that is required for neuromuscular junction integrity, is also a BMP co-receptor that confers myogenic cell-selective regulation of the BMP pathway (Yilmaz et al., 2016).MuSK binds BMPs as well as the type I BMP receptors ALK3 and 6, and enhances BMP4-induced Smad 1/5/8 phosphorylation. MuSK is expressed at the neuromuscular junction in all muscles, but is also localized extrajunctionally in soleus, suggesting that MuSK may play a novel role in this tissue. The MuSK Ig3 domain is required for high affinity BMP4 binding. To selectively manipulate the MuSK-BMP pathway in vivo, we used CRISPR/Cas9 to generate a novel model that constitutively lacks the MuSK Ig3 domain ("ΔIg3-MuSK mice"). These mice are viable and fertile. However, in ΔIg3-MuSK mice a population of muscle fibers at the periphery of soleus muscle showed hallmarks of necrosis and degeneration. In addition, WGA staining was increased in the endomysium throughout the muscle, suggesting an increase in fibrosis. Immunohistochemical fiber typing showed that a population of IIb muscle fibers is present in WT soleus but is absent in ΔIg3-MuSK mice. In agreement, high resolution SDS-PAGE showed that type IIb myosin was selectively reduced in ΔIg3-MuSK soleus. In contrast, no myofiber degeneration, loss of IIb muscle fibers or increased WGA signal was observed in the tibialis anterior of ΔIg3-MuSK mice. Taken together these data indicate that the MuSK-BMP pathway is necessary to maintain type IIb muscle fibers in soleus. Funding: Molecular Biology of Aging T32 Training Grant (NIH 5T32AG041688-07)

Program Abstract #76
Control of myonuclear positioning by skeletal muscle CIP
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The appropriate arrangement of myonuclei within skeletal muscle myofibers is of critical importance for normal muscle function, and improper myonucleus localization has been linked to a variety of skeletal muscle diseases, such as centronuclear myopathy and muscular dystrophies. However, the molecules that govern myonucleus positioning remain elusive. Here we report that muscle-specific CIP (sk-CIP) is a novel regulator of nuclear positioning. Genetic deletion of sk-CIP in mice results in misalignment of myonuclei *in vivo*, impairing skeletal muscle function and muscle regeneration, leading to severe muscle dystrophy in mdx mice, a mouse model of Duchenne muscular dystrophy. sk-CIP is localized to the centrosome in myoblasts and relocalizes to the outer nuclear envelope in myotubes upon differentiation. Mechanistically, we found that sk-CIP directly interacts with the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex and centriole Microtubule Organizing Center (MTOC) proteins to control myonuclear positioning and alignment. These findings identify sk-CIP as a long-sought muscle-specific anchoring protein that controls myonuclear position. sk-CIP could be a therapeutic target for muscle related diseases such as muscular dystrophy. CIP control of myonuclei position

Program Abstract #77

New muscle identity code components identified by TRAP control shape and size of muscle subsets in *Drosophila*

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How diversification of cell types is regulated during development to generate stereotyped tissue patterns remains a challenging question. Cell type specific orchestration of morphogenetic processes is required for instance to produce fully integrated and functional skeletal muscles. Here, we applied TRAP approach to identify gene expression signatures underlying diversification of two muscle subsets in *Drosophila.* Whentargeting Slou-positive muscle population we identified *Ptx1* as a new component of ventral muscles identity code. We also generated temporal transition profiles of TRAP-enriched genes and found that a set of genes encoding regulators of actin cytoskeleton fits into a cluster specific for lateral transverse (LT) muscles. Two of them *dCryAB* and *Gelsolin (Gel),* encoding actin-binding sHsp and actin-severing protein, respectively display LT muscle prevailing expression positively regulated by LT identity factors Apterous and Lms. In *dCryAB* mutant embryosLT muscle shapes appear irregular whereas *Gel*-devoid LT muscles occasionally develop a splitted morphology. Importantly, splitted LT muscles in *Gel* mutants are enlarged and bear a higher number of myonuclei, a phenotype phenocopied by overexpression of *duf* fusion gene. Muscle fiber splitting is a halmark of diseased muscle in Duchenne and other muscular dystrophies but how muscles get splitted is unknown. Our *Gel* mutant analyses provide evidence that an excessive myoblast fusion could result in splitting and suggest a deregulation of fusion machinery in dystrophic muscles. Altogether, we identify new muscle identity gene *Ptx1* and a new class of identity realisator genes including *Gel* whose function makes a link between muscle identity in *Drosophila* and dystrophic muscle splitting phenotype in human.

Program Abstract #78

Single-cell analysis of the muscle stem cell hierarchy identifies heterotypic communication signals involved in skeletal muscle regeneration

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Functionally heterogeneous subpopulations of MuSCs have been identified based on their expression of myogenic regulatory factors and surface markers. However, a unified organization of MuSCs and their subpopulations remains unresolved. Here, we performed temporal analysis of skeletal muscle regeneration using single-cell RNA-sequencing of myotoxin-injured adult mouse muscles. We generated over 34,000 single-cell transcriptomes spanning four muscle regeneration time-points and identified 15 distinct cell types, including a heterogeneous population of MuSCs and progenitor cells. Our analysis provides a hierarchical map of myogenic cell populations and identifies stage-specific regulatory programs that govern their contributions to muscle regeneration. Moreover, trajectory inference organized the myogenic lineage into a continuum, consisting of quiescent MuSCs, cycling progenitors, committed myoblasts, and terminally differentiated myocytes. This trajectory also revealed that MuSC stages are defined by synchronous changes in regulatory factors, cell cycle-associated, and surface receptor expression. Lastly, we built a bioinformatic model to identify over 100 candidate heterotypic communication signals between myogenic and non-myogenic cells, many involving FGFR, Notch, and Syndecan receptor families and their ligands. Syndecan receptors were involved in a large fraction of these cell communication interactions and were observed to exhibit transcriptional heterogeneity within the myogenic continuum. Using multiparameter mass cytometry (CyTOF), we confirmed that cycling MuSCs exhibit diversified Syndecan-1/2 expression, suggesting that dynamic alterations in Syndecan signaling interactions may coordinate stage-specific myogenic cell fate regulation. Our reference dataset provides a resolved hierarchical organization of myogenic subpopulations as a resource to investigate cell-cell interactions that regulate myogenic stem and progenitor cell fates in muscle regeneration.

Program Abstract #79

Multiplexed RNAscope and immunofluorescence on whole-mount skeletal myofibers and their associated stem cells

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Skeletal muscle myofibers are large syncytial cells comprising hundreds of myonuclei, and *in situ* hybridization experiments have reported a range of transcript localization patterns within them. While some transcripts are uniformly distributed throughout myofibers, proximity to specialized regions can affect the programming of myonuclei and functional compartmentalization of transcripts. Established techniques are limited by a lack of both sensitivity and spatial resolution, restricting the ability to identify different patterns of gene expression. We adapted RNAscope fluorescent *in situ* hybridization technology for use on whole-mount primary myofibers – a preparation that isolates single myofibers with their associated muscle stem cells (SCs) remaining in their niche. This method can be combined with immunofluorescence, enabling an unparalleled ability to visualize and quantify transcripts and proteins across the length and depth of skeletal muscle fibers and their associated SCs. Using this approach, we demonstrate a range of potential uses, including the visualization of specialized transcriptional programming within myofibers, tracking activation-induced transcriptional changes, quantification of SC transcriptional heterogeneity, and evaluation of SC niche factor transcription patterns.

Program Abstract #80

The Roles of β-, γ-, and α-catenins in Satellite Cell Regulation

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The significant regenerative capacity of skeletal muscle is attributed to a small population of muscle stem cells, or satellite cells (SCs). Our lab has previously shown that the conditional removal of both N- and M-cadherin from either the satellite cell or myofiber (a niche cell) diminished the myofiber-SC adhesive junction (AJ) and led to SCs entering a partial state of activation in the absence of injury. The myofiber-SC niche and cellular polarity were maintained due to expression of multiple additional cadherins, and this break in quiescence resulted in a long-term expansion of a regeneration-proficient SC pool. This raises the question of what effect complete loss of AJs has on SC regulation. β-, γ-, and α-catenins are cadherin binding partners that are required for cadherin-mediated adhesion. β- and γ-catenins play redundant roles in AJs, as do two α-catenins (αT and αE) expressed by SCs. We hypothesize that double knockouts of β- and γ-catenin or αT- and αE-catenin will fully disrupt the AJ, resulting in reduced SC numbers and impairment in regeneration in response to acute injury. To assess the role of catenins in maintaining the quiescence-promoting myofiber-SC niche, I have generated two mouse lines to assess each catenin double-knockout from the SC using a Pax7Cre^{ERT2} driver. Initial experiments reveal that conditional β- and γ-catenin double knockout mice show a significant reduction of the SC pool in skeletal muscle after 30 days after recombination, suggesting that basal adhesion mechanisms are sufficient for niche occupancy or that additional apical adhesion mechanisms exist. Further study of these mouse lines will provide key information on how cell-cell adhesion between the myofiber and SC contribute to the quiescence-promoting SC niche.

Program Abstract #81

Myogenin has distinct function in satellite cells isolated from different muscles

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Myogenic regulatory factors (MRFs; MyoD, Myf5, myogenin and MRF4) are key transcription factors in embryonic myogenesis. Myogenin was indispensable for embryonic myogenesis, while in postnatal phase, myogenin null mice were not lethal (Hasty et al. 1993; Nabeshima et al. 1993; Meadows et al. 2008), suggesting that myogenin may serve distinct function at different developmental stages. However, a role(s) of myogenin in satellite cells remains unclear. Here, we focused on satellite cell-functional

heterogeneity, *i.e.* myogenic capability may be different depending on localized environments such as the sites of skeletal muscles in adult tissues.In this study, to examine the functions of myogenin on myogenic-differentiation or myofiber-type commitment, we separately isolated satellite cells from different muscles and conducted myogenin knock-down by specific-siRNA transfection. We selected different muscle tissues such as diaphragm (Dia), gastrocnemius (Gas) and erector spinae muscle (ESM). These muscles are derived from different origin during embryonic development and locate trunk or periphery in postnatal life. Initially, we could not find the remarkable differences in the expression levels of MRFs in Dia and Gas. The expression levels of MyoD, Myf5 and MRF4 were up-regulated in ESM. However, myotube-fusion index significantly decreased in each culture. Next, we compared the expression levels of myosin heavy chain isoforms (MyHC I, IIa, IIx and IIb) as fiber-type markers. Intriguingly, MyHC IIb significantly increased in ESM, but the expression levels of other isoforms were not affected. These findings indicated that the function of myogenin on myogenic-differentiation capability of satellite cells may not varied. However, myogenin may play a role in the myofiber-type commitment in satellite cells with different muscle origins. This work was supported by JSPS KAKENHI and the grant fund from the Ito Foundation.

Program Abstract #82
Netrin-1 synthesized in satellite cells may promote fast myofiber-type commitment during myogenic differentiation phase
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We currently focus on the novel role of satellite cells in the myofiber-type regulation. The fiber types can be classified as slow- or fast-twitch muscle based on colors, contractile properties and metabolisms. Our previous studies showed that satellite cells prepared from soleus muscle (slow-fiber abundant) synthesize and secrete larger amount of semaphorin 3A (Sema3A, a multi-functional protein) than extensor digitorum longus (EDL; fast-fiber abundant), suggesting that Sema3A impacts the formation of slow-twitch myotube (Suzuki et al. 2013; Tatsumi et al. 2017). However, there were few knowledges about regulatory mechanisms about fast-twitch myotube commitment by proteins synthesized in satellite cells. In this study, we focused on the function of Netrin families (Netrin-1, 3 and 4) to regulate fast-myotube formation since those physiological significances were competed with Sema3A in neurogenesis and osteogenesis. To evaluate our hypothesis, we examined whether Netrins impact fast-twitch fiber generation through the expression profiles of Netrin families in primary satellite cell cultures and knock-down by specific siRNAs transfection. Initially, we demonstrated Netrin-1 and 4 were significantly up-regulated like a Sema3A expression pattern during myogenic differentiation phase. Next, we compared the expression levels of Netrin subtypes between soleus- and EDL-derived satellite cells. Although there were no significant differences in expression levels of Netrin-3, 4 and cell-membrane receptors of Netrins, satellite cells from EDL expressed higher levels of Netrin-1 than those from soleus. Moreover, Netrin-1 knock-down significantly reduced the expression level of fast-type myosin heavy chain, especially in type IIb. Taken together, we suggested that Netrin-1 synthesized in EDL-derived satellite cells would act as a key modulator to promote fiber-type commitment of fast muscles. This work was supported by JSPS KAKENHI and the grant fund from the Ito Foundation.

Program Abstract #83
Impact of polluted air on muscle stem cell response.
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Environmental health is a global public emergency. While it is known that pollution has a deleterious

effect on human populations, its systemic impact on different cell types throughout the body constitutes a major gap in our knowledge of pollution impact on human health. Outdoor air pollution is a complex mixture of thousands of components either primary (fossil fuel combustion, industrial processes, domestic and commercial activities…) or secondary (from the reaction of primary pollutants with sunlight and chemicals within the atmosphere). Among the most toxic pollutants, the 2,3,7,8- Tetrachlorodibenzo-p-dioxin (TCDD), an halogenated aromatic hydrocarbons commonly known as dioxin is released into the atmosphere via combustion. TCDD distributes into environmental matrices such as soil and water from which it accumulates in plant and animal tissue and, ultimately, in human tissues through food chain bioaccumulation. We have previously shown that systemic exposure of laboratory mice to TCDD leads to the induction of distinct stress responses in subpopulations of muscle stem cells. Furthermore, we identified a new mechanism for environmental stress resistance, which is mediated by the transcription factor PAX3 and induction of an "alerted" state via mTORC1 signaling.1 In order to evaluate the impact of atmospheric pollution on muscle stem cells we exposed mice in a simulation chambers to model conditions such as Beijing-type highly polluted atmosphere. We exposedPax3GFP/+ mice to polluted atmosphere for 7 days and evaluated the consequence for muscle stem cells early activation, proliferation and differentiation. Polluted air led to a bimodal cellular response with induction of mTORC1 signaling in PAX3+ stem cells and activation in PAX3- stem cells. *[1]Der Vartanian, A.et al. PAX3 Confers Functional Heterogeneity in Skeletal Muscle Stem Cell Responses to Environmental Stress. Cell Stem Cell 24, 958-973 e959, doi:10.1016/j.stem.2019.03.019 (2019).*

Program Abstract #84

miR-106b inhibition as therapeutic tool enhancing muscle regeneration in muscular dystrophies.

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Duchenne muscular dystrophy (DMD) is a genetic disorder that causes progressive muscle wasting in affected individuals and currently has no cure. Dystrophin, the defective gene product that causes DMD, plays a pivotal role in anchoring the muscle fiber to the extracellular matrix. Defects in dystrophin lead to chronic inflammation, progressive muscle degeneration, and replacement of muscle with fibroadipose tissue. Dystrophin is also expressed in satellite cells and it has been shown that the progressive loss of muscle mass might be also attributed, at least partly, to the inability of muscle stem cells to efficiently regenerate tissue loss as the result of the disease. Hence, research to restore satellite cell function has gained significant interest in recent years in order to develop new strategies to treat DMD. We have shown that miR-106b is present in a subset of highly quiescent SCs. MiR-106b is downregulated during satellite cell activation and muscle regeneration in mice. miR-106b inhibition facilities proper myogenic differentiation *in vitro* and enhances muscle regeneration *in vivo*. Moreover, we show that dystrophic mice ($DMD^{mdx)}$ display high miR-106b levels and its *in vivo* inhibition during muscle injury, via antimiR-106b intramuscular injection, contributes to enhance muscle regeneration with a significant functional recovery. RNAseq analyses in antimiR-treated dystrophic mice revealed that antimiR-106b strongly reinforce myogenesis but also others transcriptional program that positively regulate muscle regeneration such as immuno-response and epithelial-mesenchymal transition. Interestingly, we observe that miR-106b is also increased in human dystrophic SCs and its inhibition significantly increases their myogenic differentiation potential *in vitro* and *in vivo*. Therefore, we demonstrate that anti-miR106b administration is able to improve the DMD phenotype, making this molecule a compelling candidate to consider for potential clinical studies.

Program Abstract #85

Role of Laminin-2 in the Muscle Stem Cell Niche

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Merosin-deficient congenital dystrophy 1A (MDC1A) is a rare and lethal congenital disease caused by deficiency for functional Laminin 2 (LAM2) in the muscle fiber basal lamina. MDC1A is characterized by a progressive degeneration of skeletal muscle fibers and a severely impaired regenerative capacity that is paralleled by muscle stem cell (MuSC) dysfunction. We have started to interrogate the role of LAM2 for

MuSCs using novel genetic models. To this end, we generated mice with conditional MuSC specific knockout alleles of two main receptors of LAM2: Dystroglycan, and Integrin alpha7/beta 1. These models will allow us to explore the importance of the MuSC-basal lamina dialog and will provide insights into the signaling pathways downstream of LAM2. Our preliminary results suggest that MuSC dysfunction in MDC1A is largely caused by the absence of a proper anchorage to the LAM2-rich basal lamina. MuSCs deficient for LAM2 receptors display severe functional deficits and are incapable to maintain quiescence. Altogether, our work will provide a better understanding of MuSC dysfunction in MDC1A will pave the way to new therapeutic opportunities for this devastating disease.

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Program Abstract #86

Elucidation of post-transcriptional regulatory functions of Dhx36 in skeletal muscle stem cells and muscle regeneration

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Skeletal muscle regeneration relies on its resident muscle stem cells (also called satellite cells, MuSCs) which normally lie quiescently beneath muscle fiber. SCs can quickly activate and proliferate following injury, then differentiate and fuse into myofiber; meanwhile a subset of MuSCs undergoes self-renewal and returns to quiescent state to replenish the adult stem cell pool. Post-transcriptional regulation of mRNA by RNA binding proteins (RBPs) exerts critical functions in many biological processes but its importance in MuSCs remains largely elusive. Here we investigated the functional roles of a RBP and helicase, Dhx36 in MuSC and muscle regeneration. We found Dhx36 is induced during MuSC activation and its inducible deletion hampered MuSC activation and proliferation thus delayed muscle regeneration post-injury. Dhx36 is known as a helicase to unwind RNA guanine quadruplexes (rG4) structure. To elucidate the underlying mechanisms, we thus performed CLIP-seq, rG4-seq and Polysomal profiling. Combining these high throughput data led to uncovering of the binding landscape of endogenous Dhx36 and transcriptome wide rG4 formation in myoblast cells. Specifically, we demonstrated that Dhx36 can bind to diversified regions of a wide range of mRNAs, among which Gnai2 encodes a protein known to promote MuSC proliferation. Further studies showed Dhx36 can indeed bind at 5'UTR rG4 site of Gnail2 to facilitate its translation. In addition, in-depth analyses of the above high throughput data also revealed other unknown aspects of Dhx36 regulation of mRNA metabolism. Altogether our study demonstrated Dhx36 is a crucial regulator of MuSC proliferation through its diversified binding ability and versatile functions in orchestrating post-transcriptional processing of RNAs. It thus has enhanced our knowledge in the post-transcriptional regulatory mechanisms underlying muscle regeneration for future therapeutic development of treating muscle damages and diseases.

Program Abstract #87

Effect of skeletal muscle cell secretome in mesenchymal stromal cells myogenic differentiation

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It is well know that Mesenchymal Stromal Cells are capable of communicate with other tissue due to the production and secretion of the modulatory factors that regulate and induce tissue regeneration [1]. Also, they can differentiate in the injured cells and restore the lost tissue [2]. We hypothesized that after an injury, muscle cells may secrete growth factors, cytokines, or myokines and modulate the adipose-derived MSC into myogenic differentiation. Here, skeletal muscle cells were isolated and cultured on Matrigel® coated dishes. The cells were initially cultured in proliferating medium and then shifted to differentiation medium to induce muscle differentiation. Both media, were collected from cultured cells at growing differentiation conditions. MSC were harvested from mice inguinal fat pad and cultured until passage 2 with DMEM/F12 medium. Then, the cells were cultured with different media (DMEM/F12+dexamethasone, hydrocortisone and horse serum; proliferation medium; conditioned-

proliferation medium; differentiation medium; conditioned-differentiation medium; and DMEM/F12 as control) during 5 weeks to induce myogenic differentiation. Cells were photographed weekly in order to track morphological change and after 5 weeks of myogenic induction, cells were inmunostained with MyoD1 and myogenin antibodies for detecting myogenic differentiation. After 2 weeks in culture, cells cultured with both conditioned media displayed a myotube-like morphology, were more aligned, and parallel orientated, while cells cultured in the other media presented a stressed morphology and spreaded out in a free mode. Our findings suggest that subcutaneous adipose tissue is a potential source of MSCs capable to differentiate in myogenic linage under the appropriate stimulus, what make them an accessible and suitable source of cells for therapy of degenerative muscular diseases and muscle damage from trauma.

Program Abstract #88

Single Cell and Reporter Analysis of Skeletal Muscle Interstitial Cells Identifies Unique Non-myogenic Mesenchymal Cell Populations

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Non-myogenic mesenchymal cells (NMMCs), a heterogenous group of interstitial muscle-associated cells known to include pericytes and fibro-adipogenic progenitors (FAPs), are a vital component of the skeletal muscle niche. NMMCs, such as FAPs, are required for skeletal muscle development and regeneration, and are implicated in the pathologies of several muscle disorders. Since NMMCs are a heterogenous population of cells that lack clear definitions in identity and function, we performed lineage/reporter analysis on tissue sections, as well as, flow cytometry and single cell RNA sequencing (scRNA-seq) of fluorescently labeled cells sorted from the skeletal muscles of *Prx1Cre; R26-tdTomato* mice. Whole embryo and tissue-based reporter studies demonstrate that tdTomato labeled cells originating from the lateral plate mesoderm contribute directly to osteoblasts, chondrocytes, tenocytes, and muscle-associated interstitial cells within developing limbs. Further, both reporter assays and flow cytometry indicate that these tdTomato+ cells do not contribute to the myogenic, hematopoietic, or endothelial cell lineages during skeletal muscle development, homeostasis, or regeneration. Our scRNA-seq analyses indicate that these tdTomato+ muscle-associated interstitial cells are indeed NMMCs and can be categorized into eight unique cell clusters, including some populations with transcriptomic profiles that correspond to pericytes, vascular smooth muscle cells (vSMCs), FAPs, and interstitial tenocyte-like cells. We further determined that the greater FAP population, as defined by *Pdgfra* and Sca1 (*Ly6a*) positivity, in fact includes multiple sub-populations with unique transcriptomic signatures and functions. Through the identification and classification of these unique FAP subsets, we are establishing a foundation and growing our understanding of their precise impact on skeletal muscle development, regeneration and disease pathologies. Funding; NIH R01 AR057022, AR063071

Program Abstract #89

Scleraxis positive tendon cells are required for correct muscle patterning in mammalian embryo

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The skeletal muscles of tetrapod limbs are induced as progenitor cells in somite which migrate into limb bud, segregate into the muscle bellies and connect with tendon and bone. While the induction and the migration processes has been well studied, the molecular mechanisms underlie precise patterning and tissue integration process within the embryonic limb remained largely unknown. To form large number of muscle-tendon connection in reproducible manner, local tissue-tissue interactions are expected to take place. TGFb signal from muscle cells has been reported to be essential for tendon cell differentiation, however, it is not known whether tendon cells also regulate muscle cell differentiation and/or patterning. To address this issue, we took tissue specific cell ablation approach by crossing ScxCreTg and Rosa26-LSL-DTA mice. In this Scx-DTA mouse, we observed cell death specifically to Scx positive tendon lineage which resulted in severe reduction of tendon tissue. Moreover, number of muscles changed their

attachment sites in this mouse. In particular, muscles connecting body trunk and limbs, such as pectoralis major muscle, or gluteus maximus muscle have dislocated their insertion site distally, while the axial muscles are largely not affected. Dislocated muscles tend to insert to joint area where tendon cells still remained, implying that tendon cells actively attract the muscle attachment. The migration from somite to limb or the differentiation into myofiber were essentially normal, thus, tendon cells are required only for the later tissue integration process. In sum, our results suggest that the muscle patterning and attachment is regulated by the Scx-positive tendon cells in mammalian embryo, and that tissue integration of muscle and tendon is an independent step from differentiation or long range migration of muscle progenitor cells.

Program Abstract #91

Role of the TGFb-Activated Kinase 1 in muscle-resident fibro/adipogenic progenitors, a key modulator of the inflammatory environment.

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Fibrosis is a particular concern in degenerative myopathies, as it is the clinical parameter that best correlates with loss of muscle strength. Others and we have identified changes in the inflammatory milieu taking place in muscular dystrophies as a key mechanism underlying fibrosis development. Inflammatory cell-derived factors modulate the survival of Fibro/Adipocyte Progenitors (FAPs) as well as their ability to acquire a fibrogenic phenotype, at the same time limiting the activity of the myogenic progenitors driving regeneration. Conversely, activated FAPs are the main source of chemokines modulating the inflammatory environment following muscle damage. We identified TGFb-Activated Kinase 1 (TAK1) as a key signal transducer involved in these processes. TAK1 was specifically deleted in adult FAP using a Cre-ERT2 system. Damage was performed by injection of Notexin in *Tibialis Anterior* (TA) muscles of control and TAK1-KO mice. Compared to control, skeletal muscle regeneration is strongly delayed in the absence of TAK1 in FAPs. Indeed, myogenic progenitor (MP) proliferation and fusion are delay, as well as myofiber growth. Analysis of the inflammatory milieu by CYTOF -which allowed the quantification of 34 distinct surface markers- highlighted the increased infiltration of a specific type of immune cell: the eosinophils. RNAseq data from activated TAK1-KO FAPs reveals the up-regulation of a specific chemokine implicated in eosinophil migration and infiltration. Eosinophilia has been reported as a component of Duchenne Muscular Dystrophy, and these cells are particularly enriched in muscles that develop fibrosis. Thus, this chemokine could be a potential therapeutic target for muscle dystrophies. To conclude, our central hypothesis is that TAK1 in FAPs is a key-signaling node regulating cytokine secretion, and therefore governing the establishment of the inflammatory milieu in damaged muscle, which itself modulates MP fate and skeletal muscle regeneration.

Program Abstract #92

Nedd4-1 deletion impairs mitochondrial mass and muscle fiber size upon injury and regeneration

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Damaged mitochondria are degraded via a process known as mitophagy, which is functionally related to the broader autophagy pathway. Both pathways, autophagy, and mitophagy, are essential for regulating stem cell function during embryogenesis and regeneration of adult tissues, such as the skeletal muscle. Our laboratory previously shows that E3 ubiquitin ligase Nedd4-1 mediates the down-regulation of Pax7 protein levels in differentiating muscle progenitors. In A549 cells, it has been described that Nedd4-1 also interacts with LC3, important in substrate selection and autophagosome biogenesis, and p62, which bind proteins for selective autophagy. In the same model, knockdown of Nedd4-1 causes a decrease in autophagy along with generating large mitochondria. In this scenario, our purpose is to characterize the effect of deletion of Nedd4-1 in differentiation and mitochondrial remodeling, in skeletal muscle. Firstly, we analyzed the effect of muscle stem cell-specific Nedd4-1 deletion (SC-Nedd4-1 KO) on autophagy and mitochondrial mass during skeletal muscle regeneration in mice. SC-Nedd4-1 KO muscles show deficient regeneration at different days post-injury, with a prominent reduction in myofiber cross-sectional area. At the molecular level, we observed a decline in the ratio of LC3II/LC3I after injury, accompanied by a decrease in mitochondrial mass, measured by the levels of mtHSP70. In C2C12 cells, induction of

autophagy and mitophagy generates an increase in Nedd4-1 protein and RNA levels. Upon siRNA-mediated Nedd4-1 knockdown, we observed a change in the mitochondrial network morphology, resulting in a fragmented mitochondrial network, suggesting a negative effect in the mitophagy process. In conclusion, our results indicate that Nedd4-1 could have an important role in the regulation of autophagy and mitophagy during muscle regeneration. **Acknowledgments:** Fondecyt Grants 1170975 (HO) and 115106777 (VE). Doctorate scholarships Conicyt (JS).

Program Abstract #93

Deficient Skeletal Muscle Regeneration after Injury Induced by a *Clostridium perfringens* Strain Associated with Gas Gangrene

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Gas gangrene, or clostridial myonecrosis, is usually caused by *Clostridium perfringens* and may occur spontaneously in association with diabetes mellitus, peripheral vascular disease, or some malignancies but more often after contamination of a deep surgical or traumatic lesion. If not controlled, clostridial myonecrosis results in multiorgan failure, shock, and death, but very little is known about the muscle regeneration process that follows myonecrosis when the infection is controlled. In this study, we characterized the muscle regeneration process after myonecrosis caused in a murine experimental infection with a sublethal inoculum of *C. perfringens* vegetative cells. The results show that myonecrosis occurs concomitantly with significant vascular injury, which limits the migration of inflammatory cells. A significant increase in cytokines that promote inflammation explains the presence of an inflammatory infiltrate; however, impaired interferon gamma (IFN-γ) expression, a reduced number of M1 macrophages, deficient phagocytic activity, and a prolongation of the permanence of inflammatory cells lead to deficient muscle regeneration. The expression of transforming growth factor β1 (TGF-β1) agrees with the consequent accumulation of collagen in the muscle, i.e., fibrosis observed 30 days after infection. These results provide new information on the pathogenesis of gas gangrene caused by *C. perfringens*, shed light on the basis of the deficient muscle regenerative activity, and may open new perspectives for the development of novel therapies for patients suffering from this disease.

Program Abstract #94

Hox11 Gene Function in Adult Muscle Homeostasis and Regeneration

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A fundamental question in musculoskeletal growth, homeostasis, regeneration and repair is how mesenchymal/stromal cells regulate and participate in these processes. We have previously shown that *Hox11* genes are regionally expressed in the zeugopod limb (radius/ulna; tibia/fibula), exclusively in progenitor-enriched, bone marrow mesenchymal stem/stromal cells of the skeleton. We have also previously shown that *Hox11* genes are expressed in connective tissue and interstitial stroma of muscles connected to the zeugopod skeleton and that *Hox11* genes function at developmental stages to pattern the muscles attached to the zeugopod skeleton. New data shows that *Hox11* expression continues in muscle connective tissue and interstitial stroma through postnatal and adult stages and expands in response to muscle injury, consistent with a continued function in muscle tissue. Preliminary data using conditional loss of *Hox* function demonstrates that *Hox11* genes function in postnatal and adult muscle; both postnatal growth and adult homeostasis are disrupted with loss of *Hox11* function. We observe a dramatic reduction in muscle size and muscle fiber cross-sectional area after deletion of *Hox11* function. Additionally, performing lineage trace analyses using a newly generated *Hoxa11-CreER^{T2}* allele, we find that the *Hox11*-lineage remains confined to interstitial stromal cells at embryonic stages, as expected. However, at postnatal and adult stages, we observe extensive *Hoxa11*-lineage contribution directly to muscle fibers, a novel and paradigm-shifting finding that fundamentally impacts our understanding of muscle biology.

Program Abstract #95

Enhancing endogenous repair in muscular dystrophy through cellular reprogramming

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The function of muscle stem cells (MuSCs) and thereby the regenerative capacity of skeletal muscle is compromised in congenital muscular dystrophy type 1A (MDC1A). In this disease, chronic inflammation leads to alterations in the tissue microenvironment that impair the function of MuSCs up to the point where they lose their stem cell character and can no longer compensate for the continuous fiber degeneration. The discovery of cellular reprogramming revealed that through epigenetic remodeling stem cell characteristics can be induced in most cell types. We postulate that reprogramming could be used to "reset" defective MuSCs in dystrophic muscles. Here, we addressed this concept through a range of engineered mouse models of MDC1A in which we can genetically induce cyclic bouts of Yamanka factor mediated epigenetic remodeling specifically in MuSCs. We also interrogate the phenotype of dystrophic mice in which all cell types in the skeletal muscle tissue niche can be exposed to cyclic reprogramming. When comparing the tissue-wide reprogramming paradigm to the MuSC specific mouse lines, we observe differential effects on the stem cell pool and the regenerative capacity of skeletal muscle tissue. Altogether, for the first time, we interrogate whether cyclic in-vivo reprogramming can be employed for the mobilization of endogenous repair in muscular dystrophy. In the future, hope to inspire the development of novel stem cell targeted treatment approaches for these diseases. **Grant Acknowledgements:** This work is supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Fonds de recherche du Québec – Santé (FRQS), the Fondation Jean-Luc Mongrain, the Banting Research Foundation, the Canadian Stem Cell network, and the Canadian Institutes of Health Research (CIHR).

Program Abstract #96

Nitric oxide donor, Molsidomine: dose effects on endogenous nitric oxide signalling in rat muscle tissue following contusion injury

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Nitric oxide is beneficial in muscle repair, serving as a signalling molecule via 3',5'-cyclic guanosine monophosphate (cGMP) mediated pathways. Endogenously, nitric oxide production is mediated by three nitric oxide synthases (NOS), endothelial (eNOS), inducible (iNOS) and neuronal (nNOS). The long-acting nitric oxide donor, Molsidomine, has beneficial effects on skeletal muscle regeneration following contusion injury in rats. The aim of this follow-up study was to consider whether the biochemical mechanisms behind these effects could be identified and are dose-dependent. Therefore, we examined an acute treatment regime using doses of 5, 10 or 20 mg/kg of Molsidomine or placebo (4 treatment groups) administered orally immediately after and 24 hours after drop mass contusion injury or anaesthetic alone in male Wistar rats. An hour after the second treatment, rats were euthanized and gastrocnemius muscles extracted and frozen in liquid nitrogen cooled isopentane. cGMP concentrations and eNOS, iNOS and nNOS protein content in injured and uninjured muscle tissue were quantified with cGMP ELISA kits (Cayman Chemical, 581021) and Western blotting respectively. While cGMP concentrations were similar between uninjured and injured tissues (1.25±0.29 vs 0.87±0.22 pmol/ml, p=0.252) across all groups, only in injured tissue were increases in cGMP concentrations associated with increases in Molsidomine dose (r=0.41, p=0.046). In contrast, there was a negative association between iNOS expression and dose in injured tissue (r=-0.46, p=0.043) and a positive association in uninjured tissue (r=0.65, p=0.001). These findings indicate that Molsidomine treatment had dose dependent effects on iNOS expression that differed according to injury status. Also, Molsidomine dose was associated with increases in downstream signalling only in injured tissue, suggesting that the enhanced signalling response after injury with Molsidomine treatment was unrelated to iNOS protein content.

Program Abstract #97

Integration of Inflammation and Myogenesis during Skeletal Muscle Repair: The Role of the Transcription Factor Mohawk

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Efficient repair of skeletal muscle relies upon the precise coordination of cells within the satellite cell niche and innate immune cells that are recruited to the site of damage. Pro- and anti-inflammatory cytokines and chemokines are expressed by both cells of the myogenic and myeloid lineages in a tightly controlled and temporal fashion. Therefore, the genes that regulate these signaling proteins are ideal targets to enhance skeletal muscle regeneration. Previously, we identified the function of the transcription factor Mohawk (Mkx) as a repressor of myogenic differentiation and elucidated a role for Mkx in the regulation of fiber type specificity during skeletal muscle differentiation. Mkx is expressed in several tissue types, including the progenitor cells of muscle, tendon, cartilage and bone, as well as in hematopoietic stem cells that are integral in the inflammatory response following muscle injury. Based on these studies, we hypothesize that Mkx plays a key role in the early stages of muscle repair during which satellite cells and pro-inflammatory cells participate in a coordinated feedback loop to facilitate debris clearance and regeneration of muscle fibers. We found that mice lacking Mkx exhibit altered pro-inflammatory gene expression and delayed muscle regeneration. Furthermore, we found that Mkx is necessary for the timely regulation of M1 and M2 macrophage populations in response to an acute muscle injury *in vivo*. *In vitro* culture and treatment of Mkx-deficient bone marrow-derived macrophages (BMDM) with a pro-inflammatory stimuli indicated proficient capacity of $Mkx^{-/-}$ BMDM to polarize to the M1 subtype. These studies confirm the necessity of Mkx in the establishment of a robust inflammatory response *in vivo* and implicate a greater role for Mkx in efficient skeletal muscle repair.

Program Abstract #98

Tissue Engineering, Tissue Banking and Interinstitutional collaboration: the road towards regenerative medicine in Costa Rica

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Tissue Engineering combines cells, biomaterials as well as biochemical factors in order to develop viable tissues, prosthetics and organs. Along with Tissue Banking, Tissue Engineering aims at Regenerative Medicine applications. Since 2005, with a grant from the International Atomic Energy Agency and the support of health and academic institutions, Costa Rica Institute of Technology (TEC) has cared to implement in the country the culture of human skin cells for therapeutic use, and successfully supported the creation of tissue banking facilities in the region. The establishment of the Tissue Bank at the Trauma Hospital (a semiautonomous institution owned and operated by the national Insurance Institute of Costa Rica) is an example of such successful collaboration, and we currently evaluate the use of gamma irradiation for sterilization of tissues with persistent contamination after chemical disinfection. The outstanding results obtained motivated further regenerative medicine R&D in the country. TEC invests collaborative efforts in establishing techniques and technologies like 3D printing, severe plastic deformation and electrophoretic deposition, to develop biocompatible materials, scaffolds and coatings for engineered muscle skeletal tissues and maxillary bone, cultivates AD-MSC and muscle cells, and performs mechanical stimuli and electrical impedance spectroscopy techniques for eliciting cell responses like growth or differentiation. Complementarily, TEC cooperates with foreign universities and research centers in exploring the advantages provided by HULK, a photosynthetic gene therapy strategy that uses engineered microalgae as an alternative way to deliver oxygen and recombinant molecules directly in the injured area. These interdisciplinary and interinstitutional partnerships successfully boosted the regenerative medicine field in the country, aiming towards providing state-of-the-art technological solutions for several problems concerning human health.

Program Abstract #99

Estrogen-ERβ pathway controls muscle mass and regeneration in female mice

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We have recently shown that the female sex hormone estrogen is essential for comprehensively maintaining muscle function with its insufficiency affecting muscle strength and regeneration in females. However, it is still unclear whether the estrogen signaling is mediated through its receptors. To investigate the specific role of estrogen receptor β (ERβ), one of two main types of estrogen receptors, in skeletal muscle and satellite cells, we generated muscle-specific ERβ-knockout (mKO) and satellite cell-specific ERβ-knockout (scKO) mice, respectively. mKO mice displayed a decrease in fast-type dominant muscle mass and grip strength in young female mice. Young female, but not male, scKO mice exhibited impaired muscle regeneration following acute muscle injury, accompanied with defects in cell-cycle progression of satellite cells. RNA-seq analysis revealed that the loss of ERβ in satellite cells alters gene expression of extracellular matrix components, including laminin and collagen. Thus, our results indicate that the estrogen-ERβ pathway is a sex-specific regulatory mechanism that controls muscle mass and regeneration in female mice. Grants: AMED (18ek0109383h0001 and 19bm0704036h0001).

Program Abstract #100

Regulation of muscle regeneration by CREB/ CRTC transcription factors

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During muscle regeneration, numerous developmental transcriptional pathways are re-activated in satellite cells and differentiating myocytes. The cAMP response element binding protein (CREB) is required for myogenesis during development as well as for regenerative myogenesis in adulthood, and genetic activation of CREB in mice potentiates myoblast proliferation. Although cAMP signaling, CREB, and CREB target genes have been implicated in satellite cell proliferation, myoblast fusion and myogenic differentiation, it is unknown whether stimulation of cAMP signaling specifically in satellite cells could be a suitable approach to improve regenerative responses during aging and, if so, by what mechanism. We undertook genetic and biochemical approaches in mice with regenerating muscle and in isolated myocytes to begin to address these questions. We found that activation of a Gs-coupled DREADD (Designer Receptor Exclusively Activated by Designer Drugs) specifically in satellite cells potentiated proliferation after muscle injury. This indicates that cAMP signaling is sufficient to invigorate satellite cells *in vivo*. All three CRTC isoforms increase in abundance during muscle regeneration. Although CRTC2 is activated by cAMP signaling in cultured myocytes, *Crtc2* knockout mice show only a modest reduction of proliferation after injury. Instead, the closely related protein CRTC3 accumulates in nuclear fractions of early regenerating skeletal muscle, indicating that *Crtc3* may contribute to proliferative responses. We are testing this by knocking out *Crtc3* selectively in satellite cells and interrogating signaling mechanisms that may underlie differential regulation of CRTC2/ CRTC3 in myogenic cells. Our data support a model in which cAMP-inducing ligands released after muscle injury stimulate CREB/CRTC3 activity in satellite cells to support their proliferation. This work was supported by the NIH/NIAMS (AR072368 and AR059847 to RB) and the AHA (15POST25090134 to DA).

Program Abstract #101

miR-106b inhibition as therapeutic tool enhancing muscle regeneration in muscular dystrophies

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Duchenne muscular dystrophy (DMD) is a genetic disorder that causes progressive muscle wasting in affected individuals and currently has no cure. Dystrophin, the defective gene product that causes DMD, plays a pivotal role in anchoring the muscle fiber to the extracellular matrix. Defects in dystrophin lead to chronic inflammation, progressive muscle degeneration, and replacement of muscle with fibroadipose

tissue. Dystrophin is also expressed in satellite cells and it has been shown that the progressive loss of muscle mass might be also attributed, at least partly, to the inability of muscle stem cells to efficiently regenerate tissue loss as the result of the disease. Hence, research to restore satellite cell function has gained significant interest in recent years in order to develop new strategies to treat DMD. We have shown that *miR-106b*is present in a subset of highly quiescent SCs. miR-106b is downregulated during satellite cell activation and muscle regeneration in mice. miR-106b inhibition facilities proper myogenic differentiation *in vitro*andenhancesmuscle regeneration*in vivo.* Moreover, we show that dystrophic mice ($DMD^{mdx)}$display high *miR-106b*levels and it's *in vivo*inhibitionduringmuscle injury, viaantimiR-106b intramuscular injection, contributes to enhance muscle regeneration with a significant functional recovery. RNAseq analyses in antimir-treated dystrophic mice revealed that antimiR-106b strongly reinforce myogenesis but also others transcriptional program that positively regulate muscle regeneration such as immuno-response and epithelial-mesenchymal transition. Interestingly, we observe that miR-106b is also increased human dystrophic SCs and its inhibition significantly increases their myogenic differentiation potential *in vitro*and *in vivo*. Therefore, we demonstrate that anti-miR106b administration is able to improve the DMD phenotype, making this molecule a compelling candidate to consider for potential clinical studies.

Program Abstract #102
Targeted Genome Engineering to Generate Therapeutic iPSC-Derived PAX7+ Myogenic Progenitors
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Transplantation of pluripotent stem cell (PSC)-derived myogenic progenitors represent an attractive therapeutic approach to promote muscle regeneration in muscular dystrophy (MD) patients. Using conditional expression of PAX7 through the use of a lentiviral system, we have documented the robust regenerative potential of human PSC-derived PAX7-induced myogenic progenitors, which engraft extensively, promote improved muscle contractility, and seed the stem cell compartment of transplanted dystrophic muscles (Cell Stem Cell 2012;10:610). A critical aspect when considering application of this technology to patients is safety. To eliminate or minimize the risk associated with the use of lentiviral (LV) vectors that can potentially integrate into the genome, in this study we investigated whether equivalent induction of the myogenic program can be obtained by targeting PAX7 into the safe harbor locus (SHL), which provides site specific integration to overexpress transgenes without altering the endogenous gene expression level. For this, we generated a PAX7 inducible iPSC line using previously described vectors designed at targeting two different safe harbor loci: ROSA26 and AAVS1 (Stem Cell Reports 2017;8:803). Our results in iPS cells show high levels of PAX7 expression upon exposure to doxycycline. Following our *in vitro* differentiation protocol, we demonstrate that the SHL approach results in the generation of a highly expandable population of $PAX7^{+}$ myogenic progenitors that differentiate efficiently into myosin-heavy-chain (MHC)-expressing myotubes, similarly to LV-transduced $PAX7^{+}$ progenitors. Importantly, transplantation studies show that SHL PAX7-induced myogenic progenitors are endowed with muscle regenerative potential. These findings provide proof-of-principle for the potential future use of the safe harbor locus as an approach for generating therapeutic iPS cell-derived $PAX7^{+}$ myogenic progenitors.
(NIH grants R01 AR071439 and AR055299)

Program Abstract #103
Targeting Circadian Clock Regulators for Muscular Dystrophy Therapy
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The circadian clock machinery is an evolutionarily-conserved time-keeping mechanism entrained to environmental timing cues. Our previous studies have demonstrated that circadian clock regulators, including the transcription activator Bmal1 and repressor Rev-erbα, display coordinated response upon myogenic stimuli, and their concerted actions orchestrate myogenic progenitor cell (MPC) proliferation and differentiation to promote regenerative myogenesis. Using genetic and pharmacological approaches targeting Bmal1 and Rev-erba in the mdx dystrophic mouse model, here we report that Bmal1 inhibits

whereas Rev-erbα promotes muscular dystrophy disease progression, suggesting these clock regulators may represent novel targets for dystrophic disease therapy. In contrast to the mild disease phenotype in mdx mice, genetic loss-of-function of Bmal1 led to significantly elevated muscle damage in this dystrophic background, as indicated by persistently elevated creatine kinase levels, increased IgG-staining of injury area and reduced muscle grip strength. Consistent with these findings, myofiber size in Bmal1/mdx-double null mutants are substantially reduced and satellite cells cell proliferation are impaired. In contrast, loss of Rev-erbα significantly improved the dystrophic pathology of mdx mice, with reduced injury area, enhanced satellite cell proliferation and membrane integrity. As Rev-erbα is a ligand-dependent nuclear receptor amenable to small-molecule modulations, we further tested whether its pharmacological inhibition by a specific antagonist SR8278 alleviates dystrophic damage. Initial studies indicate that, consistent with its genetic loss of functions, SR8278 robustly enhanced the myogenic response in the mdx mice. Taken together, our study revealed that targeted interventions of the clock machinery may augment regenerative capacity to ameliorate muscular dystrophy. This work is supported by MDA grant 381294 and NIDDK 1R01DK112794.

Program Abstract #104
Calcitonin receptor signaling and muscle exercise
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Muscle stem cells (MuSCs) are maintained in a quiescent state, but become activated and proliferate in response to a tissue injury or intensive exercise. Molecular mechanisms underlying the maintenance of MuSC quiescence have been studied, but the relationship between the physical activity and quiescence signaling is unknown, because low activity mice (sedentary mice) were used in most of the studies. Of note, MuSCs are maintained on myofibers which repeat contraction and relaxation, suggesting that MuSCs are randomly received physical forces. In other words, MuSCs have to have a mechanism to prevent accidental or untimely activation of MuSCsagainst a certain range of skeletal muscle activity. Calcitonin receptor (CalcR)-Protein Kinase A (PKA) signaling is one of quiescence signals in MuSCs. About 15-20% CalcR-mutant MuSCs escape from the quiescence state, but they do not enter cell cycle in sedentary mice. However, when CalcR-mutant mice were housed in cages containing running wheels, the CalcR-mutant MuSCs number was increased in EDL muscle where control MuSC number was not changed. In addition, transgenic expression of the catalytic domain of PKAin MuSCs inhibited MuSCs expansion in an overloaded muscle. Taken together, our results suggest that CalcR signaling functions to suppress unprepared expansion of MuSCs by skeletal muscle activity as a quiescence signaling.

Program Abstract #105
Retinoic acid signaling regulates fate of mesenchymal progenitors during muscle regeneration and pathogenesis
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Adult skeletal muscle possesses a remarkable regenerative ability that is attributable to satellite cells. Despite such an exquisite regeneration system, skeletal muscle is occupied by fatty and fibrous connective tissue in several pathological conditions such as Duchenne muscular dystrophy (DMD). We previously reported that fatty and fibrous connective tissue originates from PDGFRα$^+$ interstitial mesenchymal progenitors. In addition to their roles in muscle pathogenesis, PDGFRα$^+$ progenitors have shown to exert positive effects on muscle regeneration. Thus, PDGFRα$^+$ progenitors have dual nature: one is to promote muscle regeneration, and the other is to contribute to pathologic changes of muscle. However, regulatory mechanism of the fate of these progenitors is largely unknown. Here we show a crucial role of retinoic acid (RA) signaling in the regulation of mesenchymal progenitor fate. We isolated PDGFRα$^+$ cells from regenerating and dystrophic muscles and compared their gene expression profiles. We found that RA signaling regulators are highly expressed in PDGFRα$^+$ cells in regenerating muscle. Analysis of RA-generating activity revealed that RA is specifically synthesized in PDGFRα$^+$ cells in regenerating muscle, but this activity is almost completely abolished in dystrophic muscle. Inhibition of RA signaling specifically reversed the promoting activity of PDGFRα$^+$ cells on satellite cell-dependent

myogenesis in co-culture. On the other hand, adipogenic and fibrogenic differentiation of PDGFRα$^+$ cells were significantly inhibited by RA supplementation. Finally, we administered RA receptor agonist to severe mouse model of DMD, D2-mdx mice. Treated mice showed significantly less fatty and fibrous degeneration, and regeneration of their limb muscle was promoted. Our study revealed that RA signaling is a critical regulator of mesenchymal progenitor fate and stimulating RA signaling would be a promising avenue for the treatment of DMD. Funding: JSPS KAKENHI #19H04063

Program Abstract #106

The Impacts of Environmental Chemicals on Telomere Length and Adverse Effects in A549 Cells

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Human exposure to environmental chemicals is associated with cardiovascular vascular disease, lung cancer, chronic respiratory disease and mortality. In this study, we investigated the exposure of one group of chemicals (oxidizing agents) can lead to adverse effects and change the telomere length as a mechanism of health effects in lung epithelial cells in cell cultures. We selected four oxidizing agents such as Potassium Bromate (KBrO3), Hydrogen peroxide (H2O2), Sodium dichromate (Na2Cr2O7) and 4-Nitroquinoline 1-oxide (4NQO) that can cause DNA damage. The compounds are suggested to be used as positive controls for the generation of DNA strand breaks and/or oxidatively damaged DNA. We measured cytotoxicity effects of cell metabolic activity, cell membrane damage and cell proliferation after 24 hours exposure to all chemicals and oxidative stress levels (ROS generation and antioxidant defense) were measured in short term and longterm exposures after 3 hours exposure to all chemicals and the effect of long-term exposure on the telomere length. The results can be summarized as (1) the short-term exposure for KBrO3 leads to low cytotoxicity and oxidative stress effect; (2) the H2O2 causes no cytotoxicity effects and moderate oxidative stress effect; (3) the Na2Cr2O7 leads to high cytotoxicity and moderate oxidative stress effect and (4) the 4-Nitroquinoline 1-oxide (4NQO) leads to high cytotoxicity and oxidative stress effects. The long-term exposure for all the chemicals induces moderate oxidative stress effect in the cells. Long term exposure to all chemicals are associated with no significant change on the telomere length was observed in A549 cells.

Program Abstract #107

Proteomic analysis of skeletal muscles reveals extracellular proteins affecting satellite cell function during aging

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During aging skeletal muscles (SMs) decrease in size and function, leading to frailty in the elderly. Satellite cells (SCs) are affected by aging resulting in decreased regenerative capacity of the muscle. The underlying molecular mechanisms still remain currently poorly understood. In this study, we systematically mapped how factors from SM are influencing SC function during aging, using a quantitative mass spectrometry strategy that enables us to monitor over 5,000 proteins in SM. We compared four different muscles from young (3 months), old (18 months) and geriatric (26 months) mice (C57BL/6). We show that different muscle types undergo different changes during aging (e.g., metabolism), while other changes, e.g., in the extracellular matrix (ECM), are conserved across SMs. Among the 322 quantified ECM proteins, 111 (34%) were significantly affected by aging in at least one muscle type. These proteins could be assigned to three main groups: inflammation, cell growth and collagen-associated. We focused on 29 ECM proteins that were consistently changed in at least two muscle types already in old mice. Among these, the SPARC-related modular calcium-binding protein 2 (Smoc2) was chosen for functional analysis, since we found it to be upregulated also in regenerating TA muscle. *In vitro* assays revealed increased MAPK (Erk1/2) signaling in MuSCs after Smoc2 treatment. *In vivo* analysis of stressed SCs by multiple injuries of TA muscles showed that Smoc2 treatment of young mice lead to a phenotype resembling aging, suggesting Smoc2 being a contributor to SM functional loss during aging. FAPs were identified as the major source of Smoc2, while accumulation of Smoc2 in the ECM could be shown to be responsible for elevated Smoc2 levels during aging. In summary, we demonstrate how the combination of an unbiased proteomic approach with functional studies allows the identification of novel regulators of SM homeostasis during aging.

Program Abstract #108

P38α MAPK coordinates the activities of several metabolic pathways that together induce atrophy of denervated muscles.

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Physiological or pathological muscle disuse/inactivity or loss of the neural-muscular junction cause muscle atrophy. Atrophy-inducing conditions cause metabolic oxidative stress in the muscle tissue, activation of the ubiquitin-proteasome and of the autophagosome-lysosome systems, enhanced removal of the damaged proteins and organelles, and loss of muscle mass and strength. The signaling pathways that control these catabolic processes are only partially known. In this study, we systematically analyzed the role of p38α mitogen-activated protein kinase (MAPK) in denervation-mediated atrophy. Mice with attenuated activity of p38α ($p38^{AF}$) are partially protected from muscle damage and atrophy. Denervated muscles of these mutant mice exhibit reduced signs of oxidative stress, decreased unfolded protein response (UPR) and lower levels of ubiquitinated proteins relative to denervated muscles of control mice. Further, whereas autopahagy flux is inhibited in denervated muscles of control mice, denervated muscles of $p38^{AF}$ mice maintain normal level of autophagy flux. Last, muscle denervation affects differently the energy metabolism of muscles in normal and mutant mice; whereas denervation appears to increase mitochondrial oxidative metabolism in control mice, it elevates anaerobic glycolytic metabolism in $p38^{AF}$ mice. Our results indicate, therefore, that attenuation of p38α activity in mice protects denervated muscles by reducing oxidative stress, lowering protein damage and improving the clearance of damaged mitochondria by autophagy.

Program Abstract #109

The aged inflammatory niche prevents stem cell contribution to muscle regeneration

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A robust and transient immune response defines the early phase of adult tissue regeneration. The persistence of this response is a feature of age-related regenerative deficits. The CC chemokine receptor Ccr2 is essential for the initial recruitment of hematopoietic lineage-derived inflammatory cells in response to tissue injury. Here, we find Ccr2 expression is induced in non-hematopoietic skeletal muscle stem cells (satellite cells; SCs) during regeneration. The increased expression of Ccr2 in SCs during early stages of adult muscle regeneration is triggered by high levels of the receptor cognate-ligands, the chemokines Ccl2, 7, and 8. Following injury and as regeneration proceeds, the levels of Ccl2, 7, and 8, in muscle and Ccr2 in SCs decreases. Consequences of chemokine-mediated Ccr2 induction are increased mitogen-activated protein kinase p38delta/gamma signaling, downstream phosphorylation of MyoD, and repression of the terminal commitment factor myogenin culminating in the inhibition of myogenic differentiation. We identified at a later stage of aged muscle regeneration accumulation of Ccr2 chemokines and macrophages. In SCs, this was associated with increased and decreased expression of Ccr2 and myogenin, respectively. Furthermore, macrophages derived from aged regenerating muscle inhibited myogenic differentiation in a Ccr2 dependent manner upon co-culture with adult SCs. Accordingly, timely Ccr2 inhibition directly to aged regenerating muscle enhanced healing. Notably, successful engraftment of adult SCs into an aged regenerating muscle host was only achieved with Ccr2 inhibitor pre-treatment. These results identify a novel inflammatory related feed-forward mechanism regulating Ccr2 expression in SCs that contributes to aged muscle regenerative decline. This work is funded by NIA (Aging – NIAR01AG051456).

Program Abstract #110

The impact of denervation on satellite cell functionality

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The neuromuscular system represents the crossroad between two tissues crucial for processes like movement or respiration. Consequently, aging of peripheral nerves and skeletal muscle fibers is marked by impaired signal transmission and muscle atrophy. Moreover, the striking regenerative capacity of skeletal muscle is declining during aging due to diminished innervation and reduced number and

functionality of satellite cells. Within this study, we want to examine how a disrupted innervation influences the overall muscle structure and function focusing on satellite cells. Since denervation is a hallmark of aging, we are analyzing satellite cell properties of mice from different age groups after sciatic nerve transection. Three weeks after denervation satellite cells are isolated and subjected to proteomics analysis, thus allowing for the identification of age-related differences between satellite cells from innervated and denervated skeletal muscles. Furthermore, we want to study how two distinct satellite cell populations contribute to cell fate in different conditions such as aging, regeneration and denervation. To this end, the reporter mouse line Rosa26-lsl-YFP; myf5-cre is used to differentiate between satellite cells based on the lineage expression of Myf5. Interestingly, as absolute numbers of both populations are decreasing with age, the ratio of committed progenitors (YFP+) to self-renewing stem cells (YFP-) is unaffected by aging in homeostasis and regeneration. To address whether innervation has a direct impact on satellite cell subpopulations we are combining a sciatic nerve transection with muscle injury in mice of different ages and are analyzing the regenerative potential of satellite cells from those muscles. In addition, parameters essential for functional innervation will be investigated during aging and under denervation conditions, including nicotinic acetylcholine receptor expression or muscle force generation.

Program Abstract #111

Dysregulated cell signalling and reduced satellite cell potential in ageing muscle

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Regulated cell signalling ensures well-orchestrated skeletal muscle cell proliferation and differentiation during fetal and postnatal growth. The satellite cells enter quiescence upon muscle growth completion by down-regulating cell signalling responsible for growth. Growth promoting signalling, however, is transiently up regulated during muscle injury and repair to activate quiescent satellite cells. Recent studies have shown spontaneous re-activation of satellite cells and FGF cell signalling in ageing muscle. Here, we examined the re-activation of Sulf1/Sulf2 enzymes that are known to regulate receptor tyrosine kinase and Wnt signalling during normal development, disease and muscle repair. Both Sulf1 and Sulf2 enzymes are highly expressed during early muscle development but show marked down-regulation during postnatal growth. This study shows gradual but progressive up-regulation of Sulf1/Sulf2 enzymes not only in the activated satellite cells but also on muscle cell membrane with increasing levels in ageing muscle. Age related progressive Sulf1/Sulf2 re-activation also correlated with the activation of receptor tyrosine kinase and Wnt signalling in ageing muscle and during accelerated ageing in progeric mouse muscles. Satellite cell culture from ageing muscle showed progressive reduction in proliferative potential, dysregulated differentiation and responsiveness to HGF. Cell culture of progeric muscle fibres also shows reduction in satellite cell number and satellite cell proliferative potential. Attenuation of activin signalling by treatment of $Ercc^{+/-}$ progeric mice with activin ligand trap over 9 weeks reduced spontaneous satellite cell activation and re-activation of cell signalling in such mice. Improved muscle recovery in these mice was also apparent from down-regulation of Sulf1/Sulf2 and Wnt signalling in treated muscles.

Program Abstract #112

Oxidative muscles have better mitochondrial homeostasis than glycolytic muscles throughout life and maintain mitochondrial function during aging

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Preservation of mitochondrial function, which is dependent on mitochondrial homeostasis (biogenesis, dynamics, disposal/recycling), is critical for maintenance of skeletal muscle function. Skeletal muscle performance declines upon aging (sarcopenia) and is accompanied by decreased mitochondrial function in fast-glycolytic muscles. Oxidative metabolism promotes mitochondrial homeostasis, so we investigated whether mitochondrial function is preserved in oxidative muscles. We compared tibialis anterior (predominantly glycolytic) and soleus (oxidative) muscles from young (3 mo) and old (28-29 mo) C57BL/6J mice. Throughout life, the soleus remained more oxidative than the tibialis anterior and expressed higher levels of markers of mitochondrial biogenesis, fission/fusion and autophagy. The respiratory capacity of mitochondria isolated from the tibialis anterior, but not the soleus, declined upon aging. The soleus and tibialis anterior exhibited similar aging-associated changes in mitochondrial

biogenesis, fission/fusion, disposal and autophagy marker expression, but opposite changes in fiber composition: the most oxidative fibers declined in the tibialis anterior, while the more glycolytic fibers declined in the soleus. In conclusion, oxidative muscles are protected from mitochondrial aging, probably due to better mitochondrial homeostasis *ab initio* and aging-associated changes in fiber composition. Exercise training aimed at enriching oxidative fibers may be valuable in preventing mitochondria-related aging and its contribution to sarcopenia. This work was supported by funds from the Board of Governors Regenerative Medicine Institute (to HSG), and by NIH R01 HL132075, P01 HL112730, and the Doris and E. Phillip Lyon Endowed Chair in Molecular Cardiology (all to RAG).

Program Abstract #113

Identification and characterization of factors involved in skeletal muscle aging

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Skeletal muscle aging is characterized by the decline in its regenerative capacity and the reduced number and functionality of muscle stem cells. One of the aims of our lab is to identify genes, which are up- or downregulated during aging, and investigate how that differential gene expression is affecting muscle stem cells and ultimately muscle regeneration. Finally, we aim to evaluate if and how those differentially expressed genes contribute to skeletal muscle aging. Therefore, we performed microarray analyses of freshly isolated muscle stem cells from 3- and 18-month old mice, thereby identifying various differentially expressed genes and micro RNAs, among them periostin and miR-5124, which were both significantly upregulated during aging of muscle stem cells. As a first analysis, we investigated their effect on differentiationof primary myoblasts. siRNA-mediated knockdown of periostin or inhibition of miR-5124 by a specific antagomir impaired differentiation, shown by smaller myotube diameters, reduced fusion index (less nuclei per myotube) and less myogenin-positive nuclei in myotubes after 3 days of differentiation. The function of periostin and miR-5124 and how they affect muscle stem cells will be analyzed by the use of floating myofibers from old mice. We will further identify the underlying signaling pathways regulated by these target genes, which contribute to skeletal muscle aging and diminished regenerative capacity in the aged. Finally, we will analyze how the regeneration of young versus old mice is affected *in vivo* after cardiotoxin-induced muscle injury in combination with siRNA-mediated knockdown or chemical inhibition of target genes. Thereby, we may provide further information to understand the mechanisms of muscle stem cell function during aging.

Program Abstract #114

An exercise-regulated long intergenic noncoding RNA improves muscle differentiation in ageing

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Long intergenic noncoding RNAs (lincRNA) are an emerging class of molecules regulating complex cellular processes. Here, we investigate the role of an exercise-regulated lincRNA in the context of muscle function and ageing. Exercise induces the expression of the lincRNA TREN1 in skeletal muscle across different species (human, rat, mouse) and genetic modification of its expression in C2C12 mouse muscle cells induces a human exercise transcriptomic landscape. In addition, skeletal muscle expression of Tren1 across a genetically diverse mouse panel (BXD) correlates with oxygen kinetics and muscle mass. Importantly, ageing reduces Tren1 expression in mouse and human skeletal muscle. GapmeR-mediated knockdown of Tren1 in C2C12 reduces myotube diameter and RNA sequencing reveals myogenesis as the most regulated pathway. Indeed, CRISPR-dCas9 mediated epigenetic upregulation of endogenous Tren1 in C2C12 induces expression of genes implicated in muscle differentiation (Myog, Myf6, Myhc), enhances myotube diameter, area and myofusion and preserves these measurements upon a dexamethasone challenge. Using 483 genotyped individuals coupled with RNA sequencing data from skeletal muscle (GTEx) we identify several putative TREN1 skeletal muscle cis-eQTLs. Performing a combination of luciferase-assays, CRISPR-mediated promoter tagging followed by ChIP and CRISPR-mediated induction of heterochromatin we identify a causal TREN1 enhancer element which harbors an allelic variant that upregulates skeletal muscle TREN1 expression. This variant associates with upper and lower body strength in aged individuals from the Helsinki Birth Cohort Study. CRISPR-dCas9 mediated

upregulation of endogenous TREN1 in muscle cells from an aged human donor (74yo) improves myotube diameter, area and myofusion together with MYOG, MYF6 and MYHC expression. In summary, our data identifies a novel exercise-regulated lincRNA whose upregulation improves the age-related decline in muscle differentiation.

Program Abstract #115
Sphingolipid depletion reverses age-associated muscle dysfunction and improves muscle regeneration
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Ceramides have previously been linked to heart disease, diabetes and cancer, but their role in aging and sarcopenia has not been explored. Here, we studied the role of ceramides in aging skeletal muscle (SKM). Upon aging, there was global accumulation of ceramides in SKM. The transcripts of ceramide synthesizing sphingolipid de novo synthesis pathway (SDNSP) were upregulated in aged human SKM. These transcript levels, especially serine-palmitoyltransferase (SPT), the rate-limiting enzyme of SDNSP, were inversely correlated with muscle weight, muscle function, and exercise capacity in BXD mouse population. Systemic treatment of aged mice with a pharmacological SPT-inhibitor for 17 weeks reduced SKM ceramide levels, and improved muscle mass, strength, coordination and exercise capacity. Unbiased gene-set enriched analysis of SKM transcriptome pointed to improved SKM regeneration and muscle stem cell (MuSC) function upon SPT inhibition. Indeed, transplantation of ceramide depleted MuSCs into aged recipients improved muscle regeneration and exercise capacity from cardioxin-induced muscle injury. Pharmacological SPT inhibition of C2C12 myoblast activated myogenesis program, highlighted by elevated expression of Myf5, Myog and Myhc2b, and increased myotube diameter. Moreover, CRISPR-Cas9-mediated SPTLC1 silencing resulted in similar activation, demonstrating cell-autonomous, SPT-dependent effect on myogenesis. In human populations we identified genetic loci associated with reduced mRNA expression of both SPTLC1 and SPTLC2, the subunits of SPT complex. In a Finnish cohort of aged individuals, the mRNA reducing alleles of both genes were associated with improved grip strength, aerobic performance, and composite senior fitness score, suggesting benefits for humans upon sphingolipid depletion. Our data demonstrate substantial improvement of muscle regeneration upon SDNSP inhibition, and suggest SDNSP inhibition as novel treatment strategy for age-related sarcopenia.

Program Abstract #116
Highly efficient differentiation of human pluripotent stem cells into transplant competent myogenic progenitors and functional myofibers
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Background: Human pluripotent stem cell-derived skeletal muscle progenitor cells (hPSC-SMPs) provide a platform for the discovery of new drugs and therapeutics for skeletal muscle diseases. Objectives: The aim of this study was to develop a simple, serum free and highly efficient protocol to generate hPSC-SMPs that robustly fuse into contractile myofibers. Results: Three unique human pluripotent stem cell (hPSC) lines (H9, 1C, M001) maintained in mTeSR1TM were seeded (3x105/cm2) into Matrigel®-coated six-well plates and subjected to a multi-step differentiation protocol. We tracked hPSC differentiation to hPSC-SMPs at distinct myogenic developmental stages for a total of 30 days. Efficient somitogenesis was observed after 96 hours of differentiation as 84 ± 9% of cells were PAX3 positive. After somite induction, cells were differentiated for a further 25 days. Cultures were then dissociated into single cells and re-seeded. Sub-cultured cells exhibited a cell surface phenotype consistent with adult human skeletal myogenic progenitors: CD56+ (98 ± 2 %), CD82+ (98 ± 1 %), CD54+ (74 ± 15%), CD10+/CD24neg (92 ± 5%) and compared to primary human myoblasts expressed high levels of PAX7 mRNA. Intramuscular transplantation of GFP-Luc labeled hPSC-SMPs into NSG mice provided evidence of engraftment

potential as assessed by in vivo bioluminescence imaging over 14 days. Linear growth kinetics of hPSC-SMPs in vitro were observed for up to 20 passages whilst maintaining extensive differentiation potential into multinucleated MyHC+ myotubes (fusion index: 63 ± 11%). Myotubes displayed organized striations, expressed sarcomeric proteins such as alpha-actinin and exhibited robust spontaneous contractions, providing evidence for the generation of functional myofibers. Conclusions: We have developed a simple, efficient, and transgene-free protocol to differentiate hPSCs into hPSC-SMPs and functional myotubes, providing a useful tool to study human skeletal muscle diseases.

Program Abstract #117

Control of C2C12 myoblast cells growth and their differentiation into myotubes by electrical impedance spectroscopy and electric field stimulation on a patterned gold electrode.

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Electric field (EF) stimulation has a direct role in cellular morphology and physiology, and it may elicit different cellular responses. The direct effects depend mainly of the type of signal applied, as well as the amplitude and frequency of the electric voltage or current applied and the type of cell studied. It has been reported that the application of the EF in a culture medium affects the migration, orientation, proliferation, differentiation and viability of cells. Stimulation may also trigger or inhibit action potentials and modify the intercellular signaling events. C2C12 myoblast is a mouse skeletal muscle cell line that can be electrically stimulated. Stimulation with low-frequency signals in the order of a few Hertz accelerates the assembly of functional sarcomeres, favors contractile activity of the cells and accelerates their differentiation. Myotubes differentiated from myoblasts can be used to monitor the activity of skeletal muscle. The control of the alignment of myotubes has a special emphasis on tissue engineering and regenerative medicine. In this research, we used a patterned gold electrode to monitoring the growth of C2C12 by electrical impedance spectroscopy, and in parallel we are exploring the characteristics of electrical signals that induce a fast differentiation of cells to myotubes. Among the signals considered for stimulation are a pure sinusoidal, triangular, square, sawtooth, exponential and pulsed electric field waves, with different frequencies and amplitudes. The amplitude of the signals is kept under 50 mV to avoid damaging the cellular membrane, and responses are described in terms of the cell viability and differentiation.

Program Abstract #120

Atypical PKCλ regulates genomic stability in muscle satellite cells

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The atypical protein kinase C λ (aPKCλ), a member of the Par complex that controls cell-polarity, has been reported to play a role in myogenic differentiation. However, the conclusive function of aPKCλ in muscle satellite cells remains unclear. We showed that activated satellite cells highly express aPKCλ protein in a polarized manner, while expression levels of aPKCλ are downregulated in mdx-derived satellite cells. To clarify the role of aPKCλ in satellite cells in muscle regeneration, we analyzed satellite cell-specific aPKCλ knockout (aPKCλ-scKO) mice. aPKCλ-scKO mice exhibited severe defects in muscle regeneration with marked fibrosis. We found that aPKCλ-scKO mouse-derived satellite cells failed to undergo proliferation, probably due to an abnormal formation of nuclear envelope and mitotic spindle, leading genomic instability. Furthermore, loss of aPKCλ in activated satellite cells resulted in a conversion into non-myogenic lineage. Thus, our results indicate that aPKCλ controls population expansion of satellite cells, at least in part, through the maintenance of genomic stability.

Program Abstract #121

Deregulation of ACTN1: a new pathogenic mechanism at stake in nemaline myopathy?

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Nemaline myopathy is a congenital neuromuscular disorder characterized by muscle weakness, fiber atrophy and presence of nemalinebodies within myofibers. However, the understanding of the underlying pathogenic mechanisms is lacking. Recently, mutations in *KBTBD13*, *KLHL40*and *KLHL41*, three substrate adaptors for the E3-ubiquitin ligase Cullin-3, have been associated with early-onset nemaline myopathies. We generated and found that skeletal muscle Cullin-3 knockout mice develop a severe myopathy and display abnormal and strong accumulation of non-muscle alpha-Actinins (ACTN1 and ACTN4). Non-muscle alpha-Actinins have been shown to act as two actin filaments crosslinkers in non-muscle cells, but they have never really been studied in muscle tissues. Our data suggest that deregulation of non-muscle alpha-Actinins in skeletal muscles is involved in the development of the disease and may represent a new pathogenic mechanism at stake in nemaline myopathy. We hypothesized that ACTN1 overexpression is sufficient to trigger nemaline myopathy. We assessed the relevance of the regulation of ACTN1 for muscle development, maintenance and for neuromuscular junction establishment. Forced expression of ACTN1 in C2C12 leads to myoblasts fusion and differentiation defects and impairment of acetylcholine receptor clustering. Our data also revealed an abnormal accumulation of ACTN1 in the muscles of NM patients with mutation in *KBTBD13* and we found that the protein localized in nemaline bodies. Our transgenic mouse model overexpressing ACTN1 under the control of skeletal muscle specific promoter will help us to test our hypothesis. Altogether, this project will help us to decipher the importance of ACTN1 deregulation for nemaline myopathy etiology and normal muscle development and function. This project is funded by the Muscular Dystrophy Association (MDA515518) and NIH (HL128457)

Program Abstract #122

Therapeutic potential of slow muscle programming for muscular dystrophy and related diseases

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In Duchenne muscular dystrophy (DMD) and murine models of the disease, fast muscle fibers are more susceptible to damage and pathological progression than slow muscle fibers, which are damage resistant and relatively spared. Promoting a slower, more oxidative muscle phenotype could protect dystrophic muscles from damage, ameliorate the dystrophic pathology and improve quality of life. Muscle plasticity can occur through exercise and/or pharmacologic approaches, but sadly many patients cannot exercise. Although chronic low-frequency stimulation (LFS) can induce fast-to-slow muscle remodeling and is well-described for sports, rehabilitation and sarcopenia, its therapeutic potential for DMD is unclear. Does LFS confer exercise-like benefits or simply exacerbate the dystrophic pathology? To address these questions, electrodes were implanted in wild type (*WT*) and dystrophic (*mdx* and *dko*) mice and hindlimb muscles wirelessly stimulated at low-frequency to characterize acute (single bout, 10 Hz, 12 h) and 'training' adaptations (10 Hz, 12h/d, 7 d/wk, 4 wk). In *WT* mice, whole genome sequencing revealed 1764 transcripts that were differentially regulated in EDL muscles after a single bout of LFS. LFS induced a fast-to-slow phenotype in TA muscles after 4 wk, with increased SDH activity and decreased myofiber diameter. Genes related to oxidative phosphorylation, fatty acid degradation and PPAR signaling were all highly enriched after training. LFS improved myofiber SDH activity independent of histopathologic alterations and conferred functional protection in both dystrophy models. The findings highlight the utility of LFS to enhance mechanistic understanding of contraction-induced muscle plasticity, and its therapeutic potential to ameliorate the dystrophic pathology, improve muscle repair, and enhance patient quality of life. Supported by the National Health & Medical Research Council of Australia (GNT1124474)

Program Abstract #123

CRISPR-engineered human stem cells for modelling Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a fatal genetic disease caused by mutations in the *dystrophin (DMD)* gene. DMD is characterised by progressive skeletal muscle wasting with accumulation of fat and connective tissue, leading to impaired muscle regeneration. Currently, there is no cure for DMD. Although mouse models have been widely used for studying DMD and testing potential drugs, the physiological differences between mice and humans may limit the clinical translation to DMD patients. There is an unmet need of physiology-relevant human cellular platforms for modelling DMD and testing therapeutic strategies. To address this challenge, we have developed novel isogenic pairs of human stem cell models for DMD. Using a novel reprogramming technology, we generated expanded potential stem cells (EPSCs) from a DMD patient fibroblast line carrying the *DMD* c.10141C>T mutation and acquired a conventional induced pluripotent stem cell (iPSC) line carrying the *DMD* c.9691C>T mutation. Using CRISPR-Cas9 genome editing, we precisely corrected these mutations to obtain two isogenic pairs of DMD and CRISPR-corrected EPSC/iPSC lines. Following a transgene-free myogenic differentiation protocol, the isogenic pairs of EPSC/iPSC were differentiated to myogenic progenitor cells (MPCs) expressing PAX7, MYOD and several known cell-surface markers as human primary muscle precursor cells. Terminal differentiation of human EPSC/iPSC-derived MPCs formed multinucleated myotubes with titin-positive sarcomeres. Full-length dystrophin expression was completely restored in the CRISPR-corrected myotubes. Interestingly, DMD-MPCs exhibited less myogenic potential and impaired fusion competence. Transcriptome analysis identified aberrant gene expression profiles correlated with the disease phenotypes. In brief, the isogenic pairs of human stem cell models provide an invaluable resource that can be exploited for modelling DMD, high-content screening, and developing cell therapies for treating DMD.

Program Abstract #124

Characterization of R-DMDdels52, a preclinical rat model of Duchenne muscular dystrophy.

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Duchenne muscular dystrophy (DMD) is an X-linked lethal muscle disorder caused by mutations in the *Dmd*gene encoding Dystrophin. Dystrophin is a crucial component of the dystrophin-associated glycoprotein complex which has a structural function in providing structural stability to the skeletal muscle, maintaining its strength and flexibility and protecting the sarcolemma from contraction-induced injury. DMD patients display degeneration of both skeletal and cardiac muscles, leading to progressive muscle wasting, loss of ambulation by the age of 10-12, and ultimately leads to cardiac and respiratory failure. Increasing use of respiratory and cardiac support in addition to corticoid treatment has extended lifespans over the past 20 years from the late teens up to the mid-30s, but these interventions do not by themselves significantly improve muscle function. Preclinical animal models have played a key role in studies of pathogenesis and treatment development, however mdx mice show a mild phenotype and do not display the gradual dramatic muscle loss observed in DMD patients. We have developed a rat model of DMD containing a deletion of exon 52 leading to complete loss of dystrophin. We have combined histology and mass cytometry experiments to characterize the impact of dystrophin mutation in this rat DMD preclinical model. R-DMDdel52 exhibit absence of dystrophin expression, leading to permanent cycles of necrosis/regeneration in various skeletal muscles and particularly dramatic in the diaphragm. R-DMDdel52 animals progressively develop an important fibrosis, display chronic inflammation, muscle fibers atrophy and type switching, finally inducing the loss of satellite cells regenerative capacities and death within around one year of age. This rat model should prove to be useful for developing therapeutic methods to treat DMD patients. *Research founded by Association Française contre les Myopathies.*

Program Abstract #125

A genome-wide CRISPR screen to identify modifiers of Dux4 cytotoxicity

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal, dominant, gain of function disease resulting in skeletal muscle paralysis that includes muscles of the face, neck, shoulders and upper arms. Mis-expression of the double homeobox 4 (DUX4) gene plays an important role in FSHD pathogenesis. DUX4 is a transcription factor, known to activate a broad variety of genes, implicated in multiple cellular processes, including pre-implantation development, RNA processing and myogenesis. How the misexpression of these genes eventually leads to muscle cell death is poorly understood. To help solve this puzzle, we created a transgenic cell line that in which a Dux4 transgene can be induced upon doxycycline admission (iDux cell line). We confirmed that in this model, DUX4 activation induces transcriptome changes that are strikingly similar to other DUX4 activation transcriptome data sets (Geng et al. 2012, Jagannathan et al. 2016, Heuvel et al. 2019). Using our iDux cells we've performed a CRISPR knockdown screen targeting all known protein coding genes in the human genome using the Brunello library from the Broad institute. Our screen has revealed some important technical considerations when perfroming genome-wide CRISPR screens, and has identified novel putative modulators of Dux4-mediated cell death. This research was supported by the Dutch Research Counsel, the FSHD Foundation and Stichting Singelswim

Program Abstract #126

Critical roles for GSK3-β and calpain-1 in mediating desmin filament loss and myofibril destruction in atrophy

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Myofibril breakdown is a fundamental cause of muscle wasting and inevitable sequel of aging and disease. We demonstrated that myofibril loss requires depolymerization of the desmin cytoskeleton, which is activated by phosphorylation. Here, we developed a mass spectrometry–based kinase-trap assay and identified glycogen synthase kinase 3-β (GSK3-β) as responsible for desmin phosphorylation. GSK3-β inhibition in mice prevented desmin phosphorylation and depolymerization and blocked atrophy upon fasting or denervation. Desmin was phosphorylated by GSK3-β 3 d after denervation, but depolymerized only 4 d later when cytosolic Ca2+ levels rose. Mass spectrometry analysis identified GSK3-β and the Ca2+-specific protease, calpain-1, bound to desmin and catalyzing its disassembly. Consistently, calpain-1 down-regulation prevented loss of phosphorylated desmin and blocked atrophy on fasting or loss of muscle innervation. Thus, phosphorylation of desmin filaments by GSK3-β is a key molecular event required for calpain-1–mediated depolymerization, and the subsequent myofibril destruction. Consequently, GSK3-β represents a novel drug target to prevent myofibril breakdown and atrophy. This project was supported by the Israel Science Foundation (grant no. 623/15) and the Israel Ministry of Science, Technology and Space (grant no. 2015-3-74) to S. Cohen. Additional funds have been received from the Russell Berrie Nanotechnology Institute, Technion, to S. Cohen.

Program Abstract #127

Mechanisms of muscle stem cell homeostasis during development of muscle contractures in cerebral palsy

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Cerebral palsy (CP), typically the result of a perinatal brain injury, is the most common cause of childhood motor disability, affecting ~1/600 neonates. The most disabling manifestations include progressive development of muscle contractures, which manifest as stiffer, shorter and atrophic muscles that lead to a reduction in passive range of motion. While an array of rehabilitation therapies and orthopedic surgeries

are used, their long-term effectiveness in preventing or reducing contractures is marginal. We have reported that the number of satellite cells (SCs) in contractured muscles of children with CP is significantly reduced. We also showed that SC-derived myoblasts from contractured CP muscles had a decreased capacity to differentiate into myotubes in vitro. Treatment of CP myoblasts with 5-Azacytidine (AZA), a DNA methyltransferase (DNMT) inhibitor and hypomethylating agent, reversed this phenotype. In this study, we show that untreated CP myoblasts proliferated and migrated faster than untreated control myoblasts from typically developing muscle. Further, we confirmed that the hyperproliferative phenotype in CP was associated with a genome-wide increase in DNA methylation levels and a transcriptional overexpression of DNA methyltransferases DNMT1, DNMT3A and DNMT3B, alongside with markers of genomic and DNA instability. AZA-dependent reduction of DNA methylation led to a reversal of this phenotype and a DNA methylation-dependent upregulation of genes coding for extracellular matrix, cell cycle inhibitors and receptor components that could support muscle stem cell renewal and/or differentiation. These observations suggest that muscle impairment in CP is in part driven by dysregulation of DNMT activity leading to a hyperproliferative phenotype that could accelerate SC pool exhaustion. The data also identify DNMTs as potential targets for regenerative treatments that could lead to prevention or reduction of muscle contractures in CP.

Program Abstract #128

GADD45A is a protective modifier of neurogenic skeletal muscle atrophy

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Skeletal muscle is a highly adaptable tissue capable of changes in size, contractility, and metabolism according to functional demands. Atrophy is a decline in mass and strength caused by pathologic loss of myofibrillar proteins, and can result from disuse, aging, or denervation caused by injury or peripheral nerve disorders. We generated a longitudinal RNA-Seq dataset from a cohort of adult C57BL/6J males subjected to tibial nerve denervation for 0 (baseline), 1, 3, 7, 14, 30, or 90 days. Using an unbiased genomics approach to identify gene expression changes across the entire longitudinal course of muscle atrophy affords the opportunity to 1) establish acute responses to denervation, 2) detect pathways that mediate rapid loss of muscle mass within the first week after denervation, and 2) capture the molecular phenotype of chronically atrophied muscle at a stage when it is largely resistant to recovery. We found that Growth arrest and DNA damage-inducible 45a (*Gadd45a*) shows one of the earliest, largest, and most sustained changes in mRNA expression following denervation. *Gadd45a*-null mice show significantly accelerated atrophy following denervation compared to wild type littermates. Myofiber cross-sectional areas for wild type and *Gadd45a*-null mice are similar at baseline, but atrophy is clearly more prominent among all myofiber types in *Gadd45a*-null mice. We hypothesize that *Gadd45a* induction following denervation represents a protective negative feedback response. We have generated conditional and epitope-tagged mouse models for *Gadd45a* to further define its role in neurogenic atrophy, and present our findings here.

Program Abstract #129

Targeting Endogenous Repair: A New Pharmacological Approach for the Treatment of Muscular Dystrophy.

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Muscular dystrophies are severe, degenerative muscle-wasting diseases frequently caused by mutations in components of the dystroglycan glycoprotein complex. In a healthy muscle, resident muscle stem cells proliferate and differentiate to regenerate the tissue upon injury. However, in many forms of muscular dystrophy, the intrinsic regenerative capacity of muscles eventually fails to compensate for the continuous asynchronous multi-focal degeneration that is paralleled by chronic inflammation and fibrosis. No disease specific pharmacological options are available for muscular dystrophy patients, and the only treatment for

some forms of the disease are corrective surgery and glucorticoids, which have a limited ability to control inflammation. Here we describe a new therapeutic avenue based on the stimulation of endogenous repair. This approach relies on peptidomimetics-derived from a small circulating hormone with the ability to stimulate muscle stem cell function, decrease fibrosis and promote vascularization. Based on the structure–activity relationship of this factor, we have generated a library of more than 150 ligand analogues with unnatural amino acids displaying increased receptor binding affinity and plasma stability.Using a mouse model of laminin deficient muscular dystrophy (MDC1A), we observed that continuous systemic administration of this novel class of compounds improves a wide spectrum of disease hallmarks, including survival, body-weight, mobility and histological features. The treatment also induced an increase in muscle stem cell number and muscle strength in functional assays. Altogether, we provide strong evidence that stimulation of endogenous repair is an efficient treatment approach for muscular dystrophy.

Program Abstract #130

Single nucleus RNA-sequencing of mouse skeletal muscle reveals transcriptional heterogeneity of myonuclei

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A skeletal muscle fiber contains hundreds of nuclei that are formed from the fusion of precursor cells during development. It has long been known that specialized subpopulations of myonuclei exist within individual fibers, such as the nuclei that reside beneath the neuromuscular junction (NMJ). Despite this, the internal transcriptional heterogeneity of muscle fibers remains largely unexplored due to the difficulties of working with multinucleated syncytia. To assess myonuclear heterogeneity, we isolated intact nuclei from mouse tibialis anterior muscles at 21 days, 5 months, and 24 months of age and performed single nucleus RNA-sequencing (snRNA-seq). We generated transcriptomes of at least 8000 nuclei for each time-point, which clustered successfully based on cell type. The majority of captured nuclei were identifiable as myonuclei, where we observed a continuum of nuclear states, with strong Type IIB and Type IIX fiber nuclear identities separated by a range of intermediate nuclei. Within 21d myonuclei specifically, we observed a trajectory of myonuclear maturation based on expression of mature myosin heavy chain genes such as Myh4 and Myh1. We observed distinct expression patterns in immature and transitional myonuclei, which provides candidate genes for driving late-stage, post-fusion myonuclear maturation, a process which remains largely unstudied. Importantly, our data also generated robust transcriptional profiles of two rare myonuclear subpopulations, the NMJ nuclei and the myotendinous junction nuclei (MTJ), and identifies numerous marker genes previously unassociated with their respective populations. Thus, our snRNA-seq results provide novel insights into previously unseen areas of skeletal muscle biology, and are a valuable resource for the study of rare, transitional, and intermediate nuclear states within myofibers.

Program Abstract #131

Novel human FKRP iPSC model and large-scale screening identify a compound for functional glycosylation of α-dystroglycan

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Dystroglycan, an extracellular matrix receptor, has essential functions in various tissues. Loss of α-dystroglycan-laminin interaction due to defective glycosylation of α-dystroglycan underlies a group of congenital muscular dystrophies often associated with brain malformations, referred to as

dystroglycanopathies. The lack of isogenic, physiology-relevant human dystroglycanopathy cell models has limited our ability to test potential drugs in a human- and neural-specific context. Here we generated induced pluripotent stem cells (iPSCs) from a severe dystroglycanopathy patient with homozygous *FKRP* mutations. We showed that CRISPR/Cas9-mediated gene correction of *FKRP* restored tissue-specific functional glycosylation of α-dystroglycan in iPSC-derived cortical neurons, whereas targeted gene mutation of *FKRP* in wild type cells disrupted this glycosylation. In parallel, we screened 31,954 small molecule compounds using a mouse myoblast line for increased glycosylation of α-dystroglycan. Using human FKRP-iPSC derived neural cells for hit validation, we demonstrated that the hit compound significantly augmented tissue-specific functional glycosylation of α-dystroglycan. Our results suggest that this is at least in part through upregulation of *LARGE1* glycosyltransferase gene expression. Taken together, isogenic human iPSC-derived physiology-relevant cells represent a valuable platform for facilitating dystroglycanopathy drug discovery and therapeutic development.

Program Abstract #132

Macrophage-mediated delivery of transgenic LIF reduces fibrosis and inflammation in mdx mice

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Dystrophic muscle function is reduced by chronic fibrosis which is driven by macrophages that occur in highly-elevated numbers in the diseased muscle. We tested whether muscle fibrosis and inflammation in mdx muscular dystrophy could be reduced by targeting therapeutic molecules to dystrophic muscle via genetically-modified macrophages that naturally home to sites of pathology. We designed a transgene in which expression of leukemia inhibitory factor (LIF) is controlled by the leukocyte-specific, CD11b promoter. Expression of the transgene (CD11b/LIF) in mdx mice reduced the accumulation of collagen type 1 in the tibialis anterior (TA) and diaphragm muscles. In addition, transgenic TAs were less stiff and more elastic, indicating significant improvements in function. CD11b/LIF mdx mice also showed reductions in macrophage numbers in the TA but not in the diaphragm at early stages of pathology. However, both the transgenic TAs and diaphragms contained fewer, pro-fibrotic, CD163+ macrophages. In part, reductions in CD163+ macrophages are attributable to autocrine effects of transgenic LIF on the macrophages. Bone marrow derived macrophages (BMDMs) from transgenic mice showed reduced expression of CD163 and the pro-fibrotic enzyme, arginase-1. Reductions in muscle macrophage numbers are also attributable to anti-chemotactic effects of the CD11b/LIF transgene. In in vitro assays, transgenic macrophages showed a reduced chemotactic response to CCL2, a potent attractant for macrophages to dystrophic muscle. In addition, transgenic muscles and BMDMs showed reduced expression of CCL2. These findings indicate that macrophages expressing a CD11b/LIF transgene reduce fibrosis by suppressing the CD163+, pro-fibrotic phenotype and by reducing macrophage recruitment, and show that genetically-modified macrophages can be used as vehicles to target therapeutic molecules to dystrophic muscle. (Supported by NIH grants F31AR071783, F32AR065845, RO1AR066036, RO1AR062579 & R21AR066817).

Program Abstract #133

Breaking down the regulation of muscle length in growth and disease

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Although the mechanisms governing muscle mass have been extensively investigated, the cellular processes regulating muscle length are relatively unexplored. Using a murine model of neonatal brachial plexus injury (NBPI), an upper extremity nerve injury that occurs during birth in humans, we have identified that neonatal denervation impairs postnatal longitudinal muscle growth, leading to contractures, or restricted joint flexibility, that mimic the disabling contractures seen in humans following NBPI and for which no curative therapies exist. The current study uses this mouse model to understand the mechanisms by which neonatal denervation impairs longitudinal muscle growth, and to identify targets to prevent contractures. Our previous work has identified that neonatal denervation does not impair myonuclear accretion, and that impaired myonuclear accretion does not limit postnatal longitudinal muscle growth. Therefore in this study we instead investigate the role of proteostasis in longitudinal muscle growth and contractures. Following unilateral surgical NBPI, protein synthesis was assessed at

the transcriptional level with RNA-sequencing and gene ontology analysis, finding upregulation of 336 genes related to muscle development. Similarly, puromycin incorporation demonstrated overall increased protein translation, consistent with increased levels of specific muscle sarcomeric proteins by western blot. Conversely, proteolysis was also elevated in denervated muscle, with increased K48-polyubuquitination, MuRF1 transcription, and 20S proteasome activity. To test whether this increased proteolysis was responsible for contractures, we treated mice post-NBPI with the 20S proteasome inhibitor, bortezomib. Bortezomib prevented contractures and restored muscle length, despite only minimal rescue in muscle mass. These findings suggest that muscle length is regulated by proteostasis and that proteolysis is a potential target for prevention of neuromuscular contractures.

Program Abstract #134

Intracellular calcium response of a primary culture of mouse skeletal muscle

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Skeletal muscle is the most abundant tissue in the body (1). In all muscle cells, contraction depends on an increase of cytosolic calcium concentration. Calcium has an extracellular concentration of 2–4 mM and a resting cytosolic concentration of 100 nM (2). In order to evaluate the calcium response of a primary culture of mouse skeletal muscle, we first established the primary culture from the femoral muscle of 6 days old mice. By using a differentiation media, myotubes were observed between 4 to 14 days. For demonstrate its differentiation, we characterize the cells using antibodies against MyoD1, Myf5, alpha-actinin 2 and myogenin. Once differentiated, myotubes were stained with Fluo-4, a calcium probe, followed by one-minute basal fluorescence and later exposed to the calcium ionophore ionomycin, that brings extracellular and intracellular calcium concentrations close together, increasing cytosolic calcium. Cells were then followed by collecting images every 6 seconds. We measured individual myotubes fluorescence using a new pipeline in Cell Profiler 3.0.0. and individual cell time-courses were plotted by Matlab. Enhancement of intracellular calcium was observed that resulted in a contraction of myotubes, giving evidence of this primary mouse muscle culture correct differentiation. This model could be useful for creating new matrices for muscle regeneration and for evaluating calcium response of toxins that affect skeletal muscle tissue. This work was supported by FEES-CONARE Project No. 741-B8-657; Project No. 1510097 Vicerrectoría de Investigación, Instituto Tecnológico de Costa Rica, and Project No.741-B8-137, Vicerrectoría de Investigación, Universidad de Costa Rica. References 1. Tedesco FS, Dellavalle A, Diaz-Manera J, Messina G, Giulio C. 2010. Repairing skeletal muscle: regenerative potential of skeletal muscle stem cells. J Clin Invest 120:11–19. 2. Kuo IY, Ehrlich BE. 2015. Signaling in Muscle Contraction. Cold Spring Harb Perspect Biol 7:a006023.

Program Abstract #135

Novel epigenetic small molecule approaches and single-cell epigenetic analysis for DMD

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Duchenne muscular dystrophy (DMD) is a muscle wasting disease caused by mutations in the gene dystrophin. Although DMD is a progressive degenerative disease, there is evidence for early, embryonic-stage defects in myogenesis and gene expression in DMD. By understanding how these early myogenic and transcriptional defects initiate and contribute to DMD pathology, we may be better positioned to identify and utilize DMD therapies. Our studies have identified one of the earliest known DMD phenotypes: a novel transcriptional trajectory of DMD human induced pluripotent stem cells (hiPSCs) undergoing myogenesis. Mining published data shows that a significant fraction of the genes deregulated

in this alternative trajectory is involved in somitogenesis, advocating for a modified muscle development program in DMD cells. We hypothesize that “epigenetic drugs”, small molecules that target chromatin modifications and transcriptional regulation, can be used to ameliorate early DMD transcriptional defects as well as improve downstream DMD pathology. Histone deacetylase inhibitors (HDACi) are one drug class that has shown promise for DMD. However, epigenetic drugs have not been broadly and systematically studied for their benefits for DMD. We performed a novel drug combination screen of an epigenetic small molecule library using DMD zebrafish. We identified a novel combination of two epigenetic drugs that rescues muscle degeneration in DMD zebrafish. To test the mechanisms of these new drugs, and to better characterize the epigenetic perturbations in DMD, we are using single-cell RNA-seq on zebrafish and human DMD iPS cells. We are testing whether epigenetic drugs correct the early transcriptional dysregulation observed in DMD cells. Our studies support the combined use of DMD zebrafish and human iPS cells as models for understanding the developmental epigenetic defects in DMD and for identifying new potential DMD drug therapies. Funded by NIH and Million Dollar Bike Ride Program.

Program Abstract #136

Generation and correction of DMD ΔEx51 mutations in mice and human cells by genomic editing

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Duchenne muscular dystrophy (DMD), an X-linked recessive disease affecting approximately 1 in 5,000 newborn males, is characterized by a progressive degeneration of skeletal and cardiac muscles. The disease is caused by the absence of a functional dystrophin protein in muscle cells which is encoded by the Dystrophin gene, consisting of 79 exons. Deletion of exon 51 (ΔEx51) is one of the most common mutations of the Dystrophin gene that causes DMD. Correction of the ΔEx51 mutation could potentially treat about 11% of patients with DMD. To study different correction strategies, we generated a new ΔEx51 mouse model and created induced DMD (iDMD) human iPSCs lacking exon 51 in the Dystrophin gene. The ΔEx51 DMD mouse model displays typical phenotypic hallmarks of DMD, including myocyte degeneration and regeneration, fibrosis, and loss of strength. Also, cardiomyocytes derived from ΔEx51 iDMD human iPSCs do not express dystrophin protein. Applying CRISPR-Cas9 gene editing technology results in exon skipping or reframing of the surrounding exons to rescue the correct open reading frame of the dystrophin transcript and consequently restore the expression of dystrophin protein. Our current progress and efforts to bring gene editing to DMD patients will be presented.

Program Abstract #137

Regulatory T cells suppress novel skeletal muscle macrophages identified by single-cell RNA sequencing

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Skeletal muscle regeneration requires the recruitment and activation of monocytes and macrophages, which intrinsically resolve when muscle homeostasis is restored. However, the failure to resolve macrophages contributes to the progression and severity of Duchenne muscular dystrophy (DMD). In previous work we demonstrated that regulatory T cells (Tregs) ameliorated the severity of muscular dystrophy by regulating M1-like and M2-like macrophage activation, including a subset of undefined macrophages. Herein, we used single-cell RNA sequencing (scRNAseq) approaches to define the phenotypic complexity and transcriptional profile of skeletal muscle macrophages in healthy and dystrophic muscle. A T-distributed Stochastic Neighbor Embedding (t-SNE) analysis of gene expression data revealed that skeletal muscle macrophages clustered into six populations. Unexpectedly, genes associated with M1 and M2 macrophages were variably and broadly expressed in all clusters, suggesting that traditional M1 and M2 markers do not distinguish functionally distinct macrophage populations in dystrophic muscle. Rather, the scRNAseq analysis indicated that the novel muscle macrophage

populations were functionally defined by unique transcriptional programs, including genes not previously associated with dystrophic muscle inflammation. We found that a population expressing high levels of galectin-3, osteopontin and CD9 was largely absent in wildtype and dystrophic muscle prior to the onset of disease, but was increased at the onset of acute pathology and further expanded when Tregs were depleted in the mdx mouse model of DMD. Our data support a new paradigm, in which unique transcriptional programs define novel macrophage populations likely adapted to the diseased muscle milieu, and Tregs act as central regulators suppressing the dynamics of macrophage activation. This work was supported by NIAID/NIH grant R21AI134657 to Villalta.

Program Abstract #138

Expansion of laminin α2 in Duchenne muscular dystrophy impairs muscle stem cell function

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Chronic muscle injury causes fibrosis of the extracellular matrix (ECM), which we hypothesize is a major contributor to failed muscle regeneration in Duchenne muscular dystrophy (DMD). We developed a decellularization method to isolate acellular muscle scaffolds to investigate the effect of ECM on stem cell function. Human iPSC skeletal muscle progenitors (SMPCs) were cultured on acellular ECM scaffolds from normal and DMD muscle. Normal scaffolds supported robust SMPC adhesion and myotube differentiation, which were inhibited by DMD scaffolds. SMPCs cultured on normal ECM upregulated integrin-ECM pathways as well as myogenesis markers. Cell stress and apoptosis genes were elevated in SMPCs exposed to DMD scaffolds. Collagen I and laminin α2 from normal scaffolds were effectively degraded by SMPCs, however, laminin α2 in the basement membrane of DMD scaffolds was resistant to remodeling. Matrix metalloproteinases were expressed in all SMPCs, suggesting that defective remodeling is inherent to the DMD ECM. Using quantitative proteomics for ECM proteins and confocal microscopy, we showed that DMD ECM resulted from increased expression and disorganization of laminins and fibronectin, and downregulation of collagens in the basement membrane, including collagen VI. We concluded that the DMD ECM impedes effective stem cell function. To further test this concept, we used a therapeutic murine model of DMD generated from overexpression of sarcospan, a protein that increases laminin-binding integrin complexes in muscle and ameliorates DMD pathology. Analysis of ECM from these mice revealed that sarcospan improved the biochemical properties of the DMD ECM, including restoration of collagen VI. Acellular scaffolds supported healthy SMPC adhesion and differentiation, permitting effective remodeling of laminin α2. Our results reveal that the DMD ECM negatively impacts stem cell function and that fibrosis is a significant barrier to effective regeneration and stem cell therapy.

Program Abstract #139

Zebrafish as a model to understand how strength training and inactivity impact Duchenne Muscular Dystrophy

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Skeletal muscle plasticity is imperative for functional adaptation to changing demands. Increased activity initiates muscle growth and decreased activity initiates muscle wasting. Although a great deal is known about the structural and functional plasticity of healthy skeletal muscle, far less is known about plasticity in diseased muscle. Here, we combined the power of the zebrafish model with the adaptability of neuromuscular electrical stimulation (NMES) to study the impact of strength training on muscle architecture, muscle function, and survival in the zebrafish model of Duchenne Muscular Dystrophy (DMD). Four NMES programs, defined by their frequency, delay, and voltage, were designed to emulate each type of strength training: endurance, hypertrophy, strength, and power. Three sessions of endurance NMES improve muscle architecture, increase swim velocity and distance traveled, and extend

survival. To determine the underlying mechanisms for these improvements, we are using a multi-level approach. Time-lapse imaging reveals less degeneration and improved regeneration of the fast-twitch muscle fibers. Endurance NMES significantly increased the number and length of branching for neuromuscular junctions. Nuclear surface area and volume also significantly increased following endurance NMES. Conversely, three days of inactivity worsen muscle architecture and decreases survival. Strikingly, inactivity followed by a single session of endurance NMES further worsens muscle architecture and obliterates its ability to regenerate. Our data provide a new methodology with which to study muscle plasticity in healthy and diseased muscle. In addition, our data clearly indicate that, at least in the zebrafish model, some strength training is beneficial whereas inactivity is deleterious for dystrophic muscle.

Program Abstract #140
Precise gene editing in primary human muscle stem cells
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CRISPR/Cas9 mediated gene editing has changed the expectations of the possibilities of gene therapy for monogenic disease. Muscular dystrophies comprise a group of more than 40 progressive debilitating diseases, all yet untreatable. Strategies to perform gene editing in any of these diseases include AAV-mediated in vivo approaches as well as combined cell and gene therapies in autologous settings. Heavy fibroblast contamination of primary muscle cell cultures obtained from diseased tissue have so far hampered attempts to approach gene editing in primary human muscle cells. We recently developed a method to purify and expand primary human muscle stem cells with 99-100% purity as determined by the myogenic marker desmin. Pure primary human muscle cells with defined mutations in *CAPN3*were genetically edited by CRISPR/Cas9 and NHEJ mechanisms. Correction efficacy was >60% in transfected cells. In conclusion, we want to demonstrate that primary human muscle stem cells are targetable by gene editing approaches if available as a pure population.

Program Abstract #141
Analysis of Gene-Edited Dystrophin Following Chronic Injury
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Duchenne Muscular Dystrophy (DMD) is the most common neuromuscular disorder with approximately 1:5000 males affected. DMD patients develop progressive muscle wasting and, as a result, become wheelchair-bound by their teenage years. DMD patients die prematurely due to early-onset dilated cardiomyopathy. DMD is caused by mutations in the dystrophin gene. These mutations result in the loss of the dystrophin protein, which is essential for maintaining sarcolemma integrity during muscle contraction. Our lab has utilized CRISPR-Cas9-mediated genomic editing technology for exon skipping or exon reframing to correct these mutations. In multiple mouse models of DMD, we have used genome editing to restore dystrophin expression in greater than 90% of muscle fibers. The truncated, but functional form of dystrophin generated by CRISPR-mediated exon skipping or reframing is sufficient to restore muscle strength and also ameliorates muscle pathology. Longitudinal studies in corrected mice indicate that gene-edited dystrophin is still expressed in muscle six months post-correction. To further validate the longevity and durability of gene-edited dystrophin, we induced chronic injury in the muscle of DMD mice by a month-long regime of downhill running. In uncorrected DMD mice, downhill running increased the number of necrotic foci in the quadriceps, gastrocnemius, and plantaris muscles. In contrast, there was no increase in the number of necrotic foci in the muscles of corrected mice. Immunohistochemistry and western blot for dystrophin showed that chronic injury did not reduce the protein levels of gene-edited dystrophin. Thus, our work demonstrates that gene-edited dystrophin is a durable and long-lasting therapeutic for DMD patients.

Program Abstract #142

Human pluripotent stem cell-derived PAX7::GFP+ cells can be engrafted to become quiescent satellite-like cells upon transplantation

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During the recent years, substantial research efforts have been put into developing stem cell therapy. Human pluripotent stem cells (PSCs), as an autologous cell source, have been successfully differentiated into myogenic cell lineages. While mouse myogenic progenitor cells were reported to have better transplantation potential than myoblasts, little is known about human myogenic progenitor cells. By using CRISPR/Cas9, we generated a PAX7::GFP reporter human embryonic stem cell line. After the step-wise myogenic specification, PAX7::GFP expressing (PAX7::GFP+) cells were isolated by FACS, and expanded *in vitro*. In *in vitro* studies, compared with the primary human myoblasts, PAX7::GFP+ myogenic progenitor cells (MPCs) exhibit comparable myogenic differentiation capability by measuring myotube formation. Their *in vivo* myogenic potentials were further evaluated by transplanting PAX7::GFP+ MPCs into satellite cell-depleted TA muscle of NSG mice. About 1 month after the transplantation, extensive human dystrophin expressing muscle fiber were detected. more importantly, some of the transplanted PAX7::GFP+ cells were found to be located in the satellite cell niche area. Single cell RNA sequencing data showed that the cellular identity of the human transplanted PAX7::GFP+ cells are in a quiescent status and displayed transcriptional characters of satellite cells. Functionally, the transplanted PAX7::GFP+ cells in the NSG mice were seen to participate in the muscle regeneration processes in re-injury models, and to repopulate in secondary recipient mice. Our findings demonstrate that the PAX7::GFP+ MPCs derived from human PSCs could be efficiently engrafted in the niche areas with transcriptional and functional characteristics similar to quiescent satellite cells. This study provides the evidence to support not only the translational application of hPSC-derived MPCs, but also the transition of hPSC-derived lineage-specified cells into postnatal stem-like cells.

Program Abstract #143

Effects of exercise on the efficacy of microdystrophin gene therapy.

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We tested the hypothesis that running wheel exercise would complement microdystrophin gene therapy in mdx mice, a model of Duchenne muscular dystrophy. Mdx mice injected with AAV9-CK8-microdystrophin or excipient were assigned to three groups: mdxRGT (run, gene therapy), mdxGT (no run, gene therapy), or mdx (no run, no gene therapy). Wildtype (WT) mice were assigned to WTR (run) and WT (no run) groups. WTR and mdxRGT performed voluntary wheel running for 21 weeks; remaining groups were cage-active. Both weekly running distance and final treadmill fatigue time for mdxRGT and WTR were similar. In contrast, mdx final treadmill time was ~4-fold less vs. both run groups. WTR and mdxRGT produced greater in vivo maximal plantar flexor torque vs. mdx at every time point after treatment, and mdxRGT vs. mdx in vitro diaphragm force-drop after an eccentric injury protocol was significantly less. Notably, rates of mitochondrial respiration from permeabilized red quadriceps fibers revealed ADP-stimulated respiration was approximately 30-40% lower in mdx than WTR, was significantly improved with gene therapy alone, and the mdxRGT combination provided the greatest bioenergetic benefit. These data demonstrate that running wheel exercise complemented AAV-CK8-microdystrophin gene therapy, improving muscle function and mitigating energetic defects in mdx mice. Funding provided by Solid Biosciences, Inc. USA

Program Abstract #145

Ribitol rescues functional alpha-dystroglycan glycosylation in a novel patient specific FKRP-associated Walker Warburg Syndrome iPS cell model

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Fukutin Related Protein (FKRP), a ribitol 5-phosphate transferase involved in O-glycosylation of alpha-dystroglycan (α-DG), is essential for the proper interaction of α-DG with laminin-α2. Therefore, dysfunctional glycosylation of α-DG results in muscle wasting diseases, known as dystroglycanopathies. Mutations in FKRP are associated with a heterogeneous spectrum of muscular dystrophies, among which, Walker-Warburg Syndrome (WWS) type A,5 (MDDGA5) represents the most severe form of congenital muscular dystrophy. WWS is a rare developmental disorder in which patients have a life expectancy of less than 3 years. Using fibroblasts from a 1-year old patient, here we established a WWS human iPS cell-derived myogenic model that recapitulates hallmarks of WWS pathology. Due to recent evidence showing the role of FKRP as a ribitol 5-phosphate transferase, we performed studies to determine whether ribitol supplementation would lead to the rescue of functional α-DG glycosylation in our human patient-specific iPS cell model. Remarkably, supplementation of ribitol partially restores functional α-DG glycosylation and laminin binding capacity of human WWS myotubes. Mechanistically, we find that FKRP residual enzymatic capacity is required for these effects as rescue is abolished in FKRP knockout PS cell-derived myotubes. These results show for the first time that ribitol can partially restore functional α-DG glycosylation in a human model of severe FKRP-related muscular dystrophy. Furthermore, it supports the use of iPS cell-derived myotubes as a trustworthy platform for *in vitro* disease modeling and drug screening.

Program Abstract #146

Straightjacket/*α2δ3* deregulation is associated with cardiac conduction defects in Myotonic Dystrophy type 1

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Cardiac conduction defects decrease life expectancy in myotonic dystrophy type 1 (DM1), a complex toxic CTG repeat disorder involving misbalance between two RNA-binding factors, MBNL1 and CELF1. How this pathogenic DM1 condition translates into cardiac conduction disorders remains poorly understood. Here, we simulated MBNL1 and CELF1 misbalance in the *Drosophila* heart and identified associated gene deregulations using TU-tagging based transcriptional profiling of cardiac cells. We detected deregulations of several genes controlling cellular calcium levels and among them increased expression of *straightjacket/a2d3* that encodes a regulatory subunit of a voltage-gated calcium channel. *Straightjacket* overexpression in the fly heart leads to asynchronous heart beating, a hallmark of affected conduction, whereas cardiac *straightjacket* knockdown improves these symptoms in DM1 fly models. We also show that ventricular *a2d3* expression is low in healthy mice and humans but significantly elevated in ventricular muscles from DM1 patients with conduction defects. Taken together, this suggests that reducing the *straightjacket/a2d3* transcript levels in ventricular cardiomyocytes could represent a strategy to prevent conduction defects and in particular intraventricular conduction delay associated with DM1 pathology.

Program Abstract #147

Functional investigation of the role of Trps1 during myogenesis and in rhabdomyosarcoma

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Tight transcriptional control is crucial for proper myogenic differentiation. Dysregulation eventually leads to diseases such as cancer. Rhabdomyosarcomas are the most common pediatric soft-tissue sarcomas, arising from proliferative myogenic progenitors that failed to complete the differentiation program. There is a high unmet clinical need to identify aberrant factors and mechanisms that drive myoblasts towards rhabdomyoblasts. Here we report a novel role for TRPS1, a GATA-like transcription factor with repressor function in regulation of normal myogenesis and deregulation in rhabdomyosarcoma. By combining loss-

and gain-of-function studies in myoprogenitor cells *in vitro* and *in vivo* we identify Trps1 as an important negative regulator of myogenic progression. Knockdown of Trps1 in muscle stem cells using floating myofiber cultures increases their proliferation while knockdown of Trps1 in cardiotoxin-injured muscles improves myogenic regeneration. Analysis of human rhabdomyosarcoma tumors revealed specific upregulation of TRPS1 in the embryonal subtype. Strikingly, depletion of TRPS1 in the embryonal RMS cell line RD improves the differentiation outcome and reduces growth of rhabdospheres, suggesting that TRPS1 is required for the maintenance of tumor differentiation block in embryonal rhabdomyosarcoma. Furthermore, we show that sustained TRPS1 levels in RD cells are a consequence of miR-1 deficiency. We are currently analyzing the tumorigenic properties of TRPS1 using *in vivo* xenografts and are identifying TRPS1 downstream targets using genome-wide approaches. In summary, our data suggest a miR-1 – TRPS1 axis which modulates myogenic progression and is dysfunctional in embryonal rhabdomyosarcoma contributing to differentiation block and tumor growth. TRPS1 might be a potential new target for rhabdomyosarcoma treatment.

Program Abstract #148
Developing a suicide gene therapy for alveolar rhabdomyosarcoma
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Rhabdomyosarcoma (RMS) is a rare childhood soft tissue cancer, whose cells resemble poorly differentiated skeletal muscle, and express a variety of myogenic proteins including the myogenic regulatory factors MYOD1 and MYOGENIN. Alveolar RMS (ARMS) represents ~30 % of RMS cases and is often diagnosed by the presence of a novel chimeric transcription factor, termed PAX3-FOXO1 or PAX7-FOXO1, generated by a chromosomal translocation. ARMS is associated with a poorer prognosis than other RMS variants, especially if expressing PAX3-FOXO1. Metastasis are commonly present at diagnosis, with the overall five year survival rate less than 30%. Due to the high degree of metastasis with ARMS, conventional therapies such as chemotherapy are generally unsuccessful, highlighting the need for novel therapeutic approaches. This study investigates a suicide gene therapy to treat ARMS, by generating an ARMS specific promoter based on the minimal human *MYOG* promoter to drive expression of HSV-TK to induce apoptosis in ARMS cells. As the *MYOG* promoter is active in healthy skeletal muscle, as well as ARMS cells, we generated and screened mutant versions lacking known transcription factor DNA binding motifs. We created a bespoke ARMS promoter that is highly active in ARMS RH30 cells but has significantly lower activity in healthy human myoblasts and myotubes. Our ARMS promoter activates an HSV-TK suicide gene that induces cell death in ARMS RH30 cells *in vitro* and in an *in vivo* mouse model. Interestingly, a subpopulation of ARMS RH30 cells are resistant to the suicide gene therapy. To investigate, we have generated multiple stable clones from RH30 cells that not only vary in resistance to the suicide gene, but in parameters including proliferation rate and cell morphology. This work is funded by a 4-year Wellcome Trust PhD Studentship (WT - 203949/Z/16/Z) in Cell Therapies and Regenerative Medicine.

Program Abstract #149
A genetic screening strategy to uncover rhabdomyosarcoma differentiation factors
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Rhabdomyosarcoma (RMS) is a malignancy of striated muscle progenitor cells, which affects primarily children and adolescents. The most common genetic alteration in RMS pathogenesis is a chromosomal translocation that fuses N-terminus of PAX3 to the C-terminus of FOXO1, resulting in a chimeric transcription factor PAX3-FOXO1 (P3F1). This fusion oncoprotein leads to a myogenic differentiation defect, promotes cellular proliferation, and oncogenic transformation. While RMS cells express several early myogenic markers such as MYOD1 and desmin, late markers of differentiation are absent and RMS cells do not fuse or form functional muscle units. In addition, RMS cells are dependent on P3F1 for growth. However, P3F1 is widely viewed as undruggable using conventional pharmaceutical approaches. Development of a targeted therapy for RMS necessitates understanding of the mechanism by which P3F1 exerts its oncogenic effects and sustains a myogenic block. To this end, we developed a

CRISPR/Cas9 phenotypic screening strategy to uncover genetic perturbations that differentiate RMS cells. Patient-derived RMS cell lines stably expressing Cas9 are infected with a pooled lentiviral single guide RNA (sgRNA) library. The pool of knockout cancer cells is then sorted based on the expression levels of endogenous myosin heavy chain (MYH), a reporter of muscle differentiation. The abundance of individual sgRNAs in each pool is quantified by Next Generation Sequencing. Genes targeted by sgRNAs enriched in the pool of cells that expresses high levels of MYH are candidate factors that support the sustenance of the aberrant myodifferentiation block in RMS. Inhibition of these factors can re-engage forward differentiation and restore lineage fates in RMS. Funding: Edward and Martha Gerry Fellowship, Friends of T.J. Foundation, Christina Renna Foundation, Michelle Paternoster Foundation For Sarcoma Research, Clark Gillies Foundation.

Program Abstract #150

Genetic Screening Identifies MYOD1 as a Potent and Specific Dependency in Rhabdomyosarcoma

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Soft tissue sarcomas are the most common extra-cranial solid tumor type in children in the U.S. Rhabdomyosarcoma (RMS) makes up nearly 50% of soft tissue sarcomas in this demographic. RMS is a round, blue cell tumor which expresses some markers of myogenic differentiation such as MYOD, myogenin, desmin, and actin. It lacks later markers of differentiation, however, and does not form myotubes or functional muscle units. As such, this cancer is thought to represent a malignancy of muscle progenitors unable to complete the myogenic differentiation program. The standard-of-care therapy for this disease has remained unchanged for the past fifty years and utilizes mechanisms of action that are not specific to this malignancy. To find elite therapeutic targets for RMS we have employed a negative-selection, domain-focused CRISPR/Cas9 screen of RMS cell lines to discover dependencies specific to this disease and characterize their potency. RMS cell lines stably expressing Cas9 are infected with pooled lentiviral gRNAs targeting exons in genes belonging to nine different functional classes. Next-generation sequencing is used to quantify gRNAs, and their relative abundance at the end of the assay defines target essentiality. Target essentialities are then compared across cancer types to assess their specificity. We have found MYOD1 to be the most potent RMS-specific target present in our screen. This finding is validated in a publicly available dataset of genome-wide CRISPR screens of cancer dependencies. As is common to many transcription factors, MYOD remains to be pharmacologically targeted. We aim to model the efficacy of MYOD inhibition in RMS in order to evaluate the support for developing anti-MYOD therapeutics. Funding: SBU School of Medicine Dean's Turner Fellowship Award, SBU MSTP T32 Training Grant, Friends of T.J. Foundation, Christina Renna Foundation, Michelle Paternoster Foundation For Sarcoma Research, Clark Gillies Foundation.

Program Abstract #151

Epigenetic tuning of miR in FAP-derived Extracellular Vesicles promotes regeneration and inhibits fibrosis in dystrophic muscles

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The functional exhaustion of muscle stem cells (MuSCs) contributes to Duchenne Muscular Dystrophy (DMD) progression by compromising the compensatory regeneration of diseased muscles. MuSCs activity is influenced by functional interactions with cell types that compose their niche, including fibro-adipogenic progenitors (FAPs) that regulate the regenerative ability of skeletal muscles in physiological

and pathological conditions. We have recently discovered that FAP-derived Extracellular Vesicles (EVs) support functional interactions with MuSCs, and contribute to the beneficial effect of HDAC inhibitors (HDACi) on DMD muscles. FAP-derived EVs mediate microRNA transfer to MuSCs, and that exposure of dystrophic FAPs to HDAC inhibitors (HDACi) increases the intra-EV levels of a subset of microRNAs (miRs), which cooperatively target biological processes of therapeutic interest, including regeneration, fibrosis and inflammation. In particular, increased levels of miR206 in EVs released from FAPs of muscles from DMD patients or mdx mice exposed to HDACi correlated with improvement of key histological parameters, such as compensatory regeneration and inhibition of fibrosis. We found that EVs from HDACi-treated dystrophic FAPs stimulated MuSC activation and expansion *ex vivo,* and promoted regeneration, while inhibiting fibrosis and inflammation of dystrophic muscles, upon intramuscular transplantation, *in vivo.* These data reveal a potential for pharmacological modulation of FAP-derived EV's content as novel strategy for local therapeutic interventions in muscular diseases. **Funding:** Italian Ministry of health #GR-2016-02362451; AFM-telethon #21657

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