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Abstract Book

Poster Presentations

Indicates the presenter is also giving a lightning talk.

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AI-designed HER2–FGFR Agonist and designed protein cocktail enhance direct myogenic reprogramming

Riya Keshri[1,2], Marc Exposit[1,3*], Mohamad Abedi[1,3*], Derrick R Hicks[1,3*], Zachary Foreman[1,2], Ashish Phal[1,2], Yen Chian Lim[1,2], Philip Barrett[2,5], Catherine Sniezek[6], Jinlong Lin[8], Thomas Schlichthaerle[1,3], Alexander J Robinson[1,2], Damien Detraux[1,2], Tung Chan Ching[1,2], Keija Wu[1], Brian Coventry[1,3], Xinru Wang[1,3], David Lee[1,3], Lemuel Chang[1], Alec S.T. Smith[2,4], David L Mack[2,4,5], Devin K Schweppe[9], Beatriz Estrada Martin[2,7], Kalina Hristova[8], Julie Mathieu[1,2,9], David Baker[1,3,10#], Hannele Ruohola-Baker[1,2#]

affiliations

1. Department of Biochemistry, University of Washington, Seattle, United States.
2. Institute for Stem Cell and Regenerative Medicine, University of Washington, School of Medicine, Seattle, United States.
3. Institute for Protein Design, University of Washington, School of Medicine, Seattle, United States.
4. Department of Neurobiology and Biophysics, University of Washington, Seattle, United States.
5.Department of Rehabilitation Medicine, University of Washington, Seattle, United States.
6. Department of Genome Sciences, University of Washington, Seattle, WA, 98105, USA
7.Departamento de Biología Celular, Universidad de Sevilla and Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain.
8.Department of Materials Science and Engineering and Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD 21218, USA
9.Department of Comparative Medicine, University of Washington, Seattle, United States.
10. Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA

Growth factor-induced receptor dimerization and activation of downstream pathways play a central role in modulating cell fate decisions. Here, we explore the potential of de novo designed synthetic ligands, termed Novokines, to reprogram cell identity by inducing proximity between novel receptor pairs. We developed, H2F Novokine, that brings together HER2 and FGFR1/2c, eliciting potent and selective signaling responses. Functionally, H2F promotes efficient reprogramming of human fibroblasts into myogenic cells and significantly enhances myofiber formation from adult patient-derived primary myoblasts, highlighting its therapeutic potential in regenerative medicine. FRET assays confirm H2F-mediated HER2-FGFR proximity, and phosphoproteomic analyses reveal activation of MAPK effectors. Notably, ERK phosphorylation is abolished in cells expressing a kinase-dead FGFR1 (K514M) mutant, confirming dependence on FGFR catalytic activity. H2F induces robust phosphorylation of the MAPK/AKT cascade while bypassing PLCγ-mediated Ca^{2+} signaling. In parallel, AI-designed minibinder cocktails that simultaneously activate FGFR1/2c or TRKA and inhibit ALK1/TGFβRII further enhance myogenic transdifferentiation, with transcriptomic analyses revealing upregulation of muscle regulators MYOG, ID3, MYC , HGF, and myokine FIBCD1. These findings demonstrate that synthetic receptor pairings can rewire intracellular signaling to drive lineage-specific outcomes. By enabling tunable, pathway-selective receptor engagement, Novokines provide a powerful, programmable platform for modeling skeletal muscle differentiation, studying metabolic signaling networks, and advancing regenerative strategies for muscle repair and sarcopenia.

Evaluating Drug-Responsive Gene Editing Constructs for *In Vivo* Gene Therapy of Duchenne Muscular Dystrophy

Bianca Druta[1,2,3,5], Quynh Nguyen [1,2,3,4,5], Nuoying Ma [1,2,3,5], Stephen D. Hauschka [2,3,4,5], and Niclas E. Bengtsson [1,2,3,5]

[1] Department of Neurology, [2] Senator Paul D. Wellstone Muscular Dystrophy Specialized Research Center Seattle, [3] Institute for Stem Cell and Regenerative Medicine, [4] Department of Biochemistry, [5] University of Washington, Seattle, WA.

Duchenne Muscular Dystrophy (DMD) is a devastating muscle degenerative disease caused by mutations in the dystrophin gene. One function of dystrophin is to stabilize and protect muscle cells from injury during contraction. Lack of functional dystrophin causes recurring muscle damage resulting in muscle necrosis and replacement of fibers with fibrotic tissue and fat. Experimental in vivo gene editing therapies aim to restore functional dystrophin protein expression through AAV-mediated CRISPR/Cas9 gene editing to correct causative mutations in the dystrophin gene. While these approaches have shown great promise, significant immunological concerns arise with the continuous expression of bacterial editing components, like Cas9. This project aims to address these complications by developing a system of drug-responsive and tunable gene editing that can toggle expression of editing components on and off. Here we present data from ongoing cell culture studies where we screened two classes of RNA elements capable of regulating expression of genes integrated in AAV expression constructs: riboswitches and pA regulators. Our results show that pA regulators clearly outperform riboswitches. Further *in vitro* tests helped identify a top pA regulator candidate, exhibiting a favorable dose response curve to the drug Tetracycline (Tet) at clinically relevant doses. Current studies are focused on adapting top pA regulators for temporal regulation of AAV-mediated dystrophin gene editing activity in the *mdx* mouse model of DMD. Overall, this tunable editing system is showing great promise for reducing risks associated with *in vivo* AAV-mediated gene therapy for DMD, presenting a safer and more efficacious approach to gene therapy.

Hyper- and Hypocontractility: A Functional Assessment of β-Myosin Mutation E525K in Dilated Cardiomyopathy Models

Kieran Fruebis, Kalen Robeson, Rachelle Soriano, Tim McMillen PhD, Kirsti Kooiker PhD, Jennifer Davis PhD, and Mike Regnier PhD

Introduction: Dilated Cardiomyopathy (DCM) is a leading cause of heart failure worldwide. Many genetic mutations have been implicated in DCM, including the MYH7 mutation E525K.

Objectives: Previous studies of isolated E525K myosin suggest it stabilizes the off state of myosin but also increases the intrinsic ATPase rate of the S1 head. This project aims to elucidate the mechanisms of how E525K drives disease progression in cells and tissues.

Methods: Engineered stem cell lines with the E525K mutation were differentiated into cardiomyocytes. Patterned cardiomyocytes were imaged during contraction to assess morphology, contractility, and calcium transients, and used to isolate myofibrils for organelle-level analysis. Cardiomyocytes were also combined with stromal cells and ECM to form Engineered Heart Tissues for tissue-level measurements.

Results: E525K mutants showed 65% reduced sarcomere shortening in isolated cardiomyocytes and 39% decreased maximal force in engineered heart tissues. Interestingly, myofibrils from the E525K mutant line showed 45% increased peak force and slightly elevated force at physiological calcium levels. Mutant cells also had fewer, smaller, less organized sarcomeres, with no differences in calcium handling compared to WT.

Conclusion: This study demonstrates that E525K myosin, though clinically linked to DCM and hypocontractility, produces both hyper- and hypocontractile effects. These findings highlight the mechanistic variability of DCM causing mutations, posing a clinical challenge for myosin-targeted therapies.

Title: A Humanized Vessel-on-a-chip Model to Understand Fibroblast-Endothelial Crosstalk

Authors: Likitha Nimmagadda, Abby Nagle, Silvia Marchianò, Ying Zheng, Jennifer Davis

Abstract:

Background: Cardiovascular diseases (CVDs) are the leading cause of deaths worldwide, equating to approximately 800,000 fatalities each year in the US alone. Cardiac Fibroblasts (CFs) play a critical role in myocardial remodeling, particularly in relation to vessel stability and branching. Recent data from the Davis lab suggest indeed that the CF activation state, morphology, and spatial position influence coronary vasculature in a disease-dependent manner; however, *little is known about how CFs interact with endothelial cells (ECs) in cardiac diseases*.

Approach: In vivo modalities for studying CF-EC interactions are limited by the inability to lineage trace CF activation transitions in situ, necessitating a more robust and easily manipulable in vitro model to fill this gap. We propose the use of a Vessel-on-a-chip (VoC) platform to study the CF-EC interplay in a 3D, high-throughput format, with specific control of biophysical cues using human-induced pluripotent stem cell (hiPSC) derived CFs. With the use of FRET vinculin tension sensor (VnTs) derived CFs, the influence and spatial heterogeneity of CF adhesion forces can also be probed through fluorescence imaging.

Results: WT and FRET VnTs hiPSCs are differentiated into CFs and seeded in a bulk collagen gel with ECs seeded within a patterned lumen. Gravity-driven flow is established, and the VoCs are cultured for seven days before being fixed and imaged. CF spatial positions, morphologies, and adhesion forces are analyzed through FIJI, MATLAB, and Ilastik.

Conclusion: This project will bring a novel understanding of how fibroblast plasticity impacts vasculature in CVD through a multidisciplinary approach.

Optimization of FKRP Gene Therapy Vectors for the Treatment of Limb-Girdle Muscular Dystrophy R9

Pranali P. Mistry[1,2], Hichem Tasfaout[1,2], Stephen D. Hauschka[2,3], Melissa J. Spencer[4], Jeffrey S. Chamberlain[1,2,3]

[1] Department of Neurology, University of Washington School of Medicine, Seattle, Washington, USA.

[2] Senator Paul D. Wellstone Muscular Dystrophy Specialized Research Center, University of Washington School of Medicine, Seattle, Washington, USA.

[3] Department of Biochemistry, University of Washington School of Medicine, Seattle, Washington, USA.

[4] Department of Neurology, Broad Stem Cell Research Center, University of Los Angeles, Los Angeles, California, USA.

Limb-Girdle Muscular Dystrophy R9 (LGMDR9) is an autosomal recessive genetic condition involving progressive muscle degeneration in the shoulders, arm, hip, trunk, and leg muscles. The disease is associated with mutations in Fukutin-Related Protein (FKRP) gene, which is involved in the glycosylation of α-dystroglycan—a laminin-binding protein known to preserve myofiber integrity. Although adeno-associated viral (AAV) vector-mediated FKRP approaches have entered clinical trials, there are concerns with its efficacy, emphasizing the need for vector optimization.

In this study, we developed and tested a series of AAV-based gene therapy constructs to introduce FKRP and restore muscle function in striated muscle. The vector panel included constructs with the native FKRP coding sequence, two different codon-optimized sequences (CO1 and CO2), and ones encoding dual copies of FKRP. Expression was mediated by the striated muscle-specific CK8e promoter or the novel skeletal muscle-specific RC3 promoter. Using the myotropic AAV-MYO1 capsid, we delivered the construct either locally or systemically to a mouse model of LGMDR9. To assess controlled FKRP expression in hepatic tissue and potential secretion into circulation, we also utilized a liver-specific ApoEhAAT promoter.

Our proof-of-concept results demonstrated marked improvements in muscular dystrophy phenotypes, including increased muscle force in the tibialis anterior and diaphragm. These findings highlight that low doses of myotropic AAV vectors can reduce muscle pathology arising from FKRP deficiency. Ongoing studies are focused on comprehensive histological, biodistribution, and molecular analyses to assess the therapeutic potential of such optimized FKRP gene therapy vectors.

Identification of a Unique Cellular and Mitochondrial Antioxidant of Dietary Origin

G. A. Nagana Gowda[1,2], Wentao Zhu,[1,2] Vadim Pascua[1,2], Tim McMillen[2], Rong Tian[2], and Daniel Raftery[1,2,3]

[1]Northwest Metabolomics Research Center and [2]Mitochondria and Metabolism Center, Anesthesiology and Pain Medicine, University of Washington, Seattle, WA 98109, [3]Fred Hutchinson Cancer Center, Seattle, WA 98109

Abstract

Ergothioneine (ERG), a naturally occurring antioxidant of dietary origin, is gaining increasing attention due to its crucial roles in human health and diseases. Despite its significance, ERG is rarely detected in biospecimens by mass spectrometry (MS) and, to date, had not been characterized by nuclear magnetic resonance (NMR) spectroscopy, two widely used techniques in metabolomics. In this study, we investigated human plasma, whole blood (WB), and red blood cells (RBC), as well as mouse blood and tissues combining NMR, LC-MS and ratio analysis techniques. The results demonstrate the ability of simple 1D ^{1}H NMR to routinely identify and quantify ERG in various biological samples. The levels of ERG vary widely and depend on the type of biological sample, with human RBC exhibiting remarkably high concentrations, often exceeding 1.5 mM. The average levels of ERG in human plasma, WB, and RBC were in the ratios of 1:70:140, respectively. Moreover, ERG levels showed a linear correlation between plasma and WB (R^2 = 0.59), plasma and RBC (R^2 = 0.75), and WB and RBC (R^2 = 0.98). In mice, ERG levels exhibit a distinct whole-body distribution, with the average levels in mouse skeletal muscle, brain, heart, kidney, and liver in the ratios of 0:1:10:35:45, respectively. The demonstration of surprisingly high levels of ERG in biological samples using ^{1}H NMR opens new avenues for its analysis using both NMR and MS methods to explore its roles in human health and diseases, as a part of routine global or targeted metabolomics studies.

Characterizing Mutations in Glycogen Storage Disease Type VII

Authors: Emily Li, Lauren Salay Justin M. Kollman

Glycogen Storage Disease VII (GSDVII) is a genetic disorder arising from an autosomal recessive trait that affects approximately 1 in 20,000-43,000 live births. Individuals diagnosed with GSDVII experience exercise intolerance, muscle cramps, myoglobinuria, and rhabdomyolysis. GSDVII is caused by a deficiency in muscle phosphofructokinase (PFKM). PFKM catalyzes the rate limiting step in glycolysis, the phosphorylation fructose-6-phosphate. It governs the flux of carbon through catabolic pathways and is thus tightly regulated by several metabolic intermediates and interactions with other glycolytic factors. While PFKM deficiency has been implicated in GSDVII, the nature of the deficiency is not defined. Several missense mutations in PFKM are associated with GSDVII. Many of these missense mutations occur distal to the active site, suggesting that mechanisms other than catalytic deficiency contribute to PFKM deficiency in GSDVII. Here, I expressed and purified naturally occurring disease-associated mutants of PFKM. I characterized the catalytic activity of these disease variants using a coupled enzyme assay and examined the influence of pH on regulation. Furthermore, I used negative stain electron microscopy and mass photometry to understand the consequences of these mutations on PFKM structure and oligomeric states. These findings will provide mechanistic insight into PFKM deficiency in GSDVII.

Title: Novel Aged Skeletal Muscle Constructs as a Model to Investigate Mitochondrial Function

Authors: Christian M. Paulos, David L. Mack, David J. Marcinek, Matthew D. Campbell

Introduction: Prior research using preclinical rodent models has identified dysfunction of mitochondrial substrate utilization following contraction in aged muscle; Investigation of substrate utilization following contraction in human muscle is technically challenging, and it is unclear how mitochondria utilize substrates following exercise contributes to the development of sarcopenia.

Objectives: The primary objectives of this study is to investigate the mitochondrial function in three-dimensional human muscle tissue (3D-EMT) and characterize aging's effects on longitudinal contractile training of 3D-EMTs.

Methods: To investigate these aims, we collaborated with the Study of Muscle Mobility and Aging (SOMMA) to develop naturally aged (70+) and young (35-69) myoblasts differentiated into 3D-EMTs. 3D-EMT was stimulated to contract using a Magnetic Analyzer for engiNeered Tissue ARRAY (MantARRAY, Curi Bio). Following contraction, we measured mitochondrial respirometry, metabolomics, transcriptomics, and metabolic flux to compare metabolic response to contraction to muscle contraction.

Results: Results show that aged 3D-EMTs have significantly less force, greater fatigue resistance, lower contraction and relaxation velocities compared to young 3D-EMTs. Aged 3D-EMT also have lower mitochondrial respiration during LEAK state and decreased oxidative phosphorylation capacity compared to young 3D-EMT.

Validation of a Novel Phenotype for Classifying Hypertrophic Cardiomyopathy-Associated *MYH7* Variants of Uncertain Significance

Sruthi Balasubramanian, Clayton E. Friedman, Alex Loiben, Wei-Ming Chien, Kai-Chun Yang

Hypertrophic cardiomyopathy (HCM) involves abnormal thickening of the cardiac left ventricle which can lead to heart failure and sudden cardiac death. Pathogenic variants in *MYH7,* the gene that encodes the motor protein β-MHC, account for ~40% of HCM cases. However, ~75% of known *MYH7* variants are of uncertain significance (VUS), leading to an unmet need to understand if they are pathogenic or benign. A multiplexed β-MHC abundance assay from our lab accurately distinguishes pathogenic and benign *MYH7* variants in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), enabling VUS classification. Although most pathogenic variants displayed reduced β-MHC abundance, a subset showed increased levels, which could involve a distinct disease mechanism. To test that hypothesis, here we functionally assess several pathogenic variants using orthogonal assays to validate the β-MHC abundance assay and to better understand the underlying disease mechanism. Heterozygous variants that showed increased β-MHC abundance (p.Lys865Glu, p.Leu881Met, and p.Ser851Phe) were knocked into the endogenous *MYH7* locus using CRISPR/Cas9 editing to generate transgenic hiPSC lines. These clonal hiPSCs are differentiated to cardiomyocytes and are evaluated for β-MHC abundance using flow cytometry, cell size using confocal microscopy, gene expression using RNA-seq, and contractility via traction force microscopy. These variants are expected to show increased β-MHC abundances and contractility, and expression of genes such as *NPPA* and *NPPB* relative to wildtype. Studying these phenotypes will help improve the utility of β-MHC abundance assays for screening VUS, informing earlier medical interventions to improve health outcomes for patients that develop HCM.

Presenting Author: Benjamin R Pryce, Acting Instructor

Title: Intercellular Signaling within the Muscle Microenvironment Controls Muscle Wasting in Cachexia

Authors: Alexander Oles, Victoria Spadafora, Erin E. Talbert, Martin J. Romeo, Silvia Vaena, David J. Wang, Denis C. Guttridge, **Benjamin R. Pryce**

Cancer cachexia is a debilitating condition characterized by weight loss due in large part to muscle wasting. Cachexia has traditionally been described as a disorder of systemic inflammation, with tumor secreted factors speculated to drive tissue wasting. More recently, we and others have found that skeletal muscle is locally inflamed in cachexia. This inflammation was characterized by an increase in neutrophils and macrophages and was due in part to nuclear factor κB (NF-κB) activity in multiple cells, including satellite cells, myofibers, and fibro-adipogenic progenitors (FAPs). Single cell sequencing revealed that there was an upregulation of inflammatory cytokines in these macrophages, most notably IL-1β. Ablation of these inflammatory macrophages partially prevented cachexia in tumor bearing mice. Interestingly, we found that FAPs express high levels of the IL-1β receptor, IL-1R1. We also observed a unique transcriptional profile of FAPs in cachectic muscle, with a significant downregulation in the **S**ecreted **P**rotein and **R**ich in **C**ysteine (SPARC) transcript. This was of interest, as SPARC has been shown to be necessary for maintaining muscle growth and function. Treatment of cultured FAPs with IL-1β led to downregulation of SPARC. We believe this indicates that inflammatory macrophages signal to FAPs through IL-1β to downregulate SPARC expression in cachectic muscle. Moving forward, we will test whether over-expression of SPARC or inhibition of IL-1β signaling can reverse the cachectic effects caused by muscle inflammation.

Title: Establishing Cardiac Fibroblasts as Orchestrators of Cardiac Decompensation in Familial Dilated Cardiomyopathy

Authors: Sasha Smolgovsky, Amy Gifford, Abdel Ibrahim, Sebastian Lorete, Farid Moussavi-Harami, Darrian Bugg, and Jennifer Davis

Introduction: Cardiac fibrosis is an established hallmark of heart failure and strong predictor of negative outcomes in patients with dilated cardiomyopathy (DCM). Cardiomyocyte-targeted drugs successfully restore contractility, but fail to completely recover cardiac function. Cardiac fibroblasts maintain the extracellular matrix and myocardium in the resting and diseased heart - whether fibroblasts crosstalk with immune cells to perpetuate fibrosis in familial DCM remains unknown.
Hypothesis: Fibroblast mechanosensation of cardiomyocyte dysfunction promotes fibroblast expansion and immune cell recruitment, resulting in fibrosis-driven cardiac decompensation.
Methods: Cardiac function was assessed in C57BL/6J mice expressing the patient-derived D230N-tropomyosin mutation (D230N) and littermate controls by echocardiography at 1 and 2 months of age. Picrosirius red-fast green stain of cryosections was used to measure fibrosis. The abundance of PDGFRα^+ fibroblasts and cardiac immune cells were measured by immunofluorescence.
Results: D230N mice present with a canonical DCM phenotype with significantly decreased systolic function, increased left ventricular chamber dilation, and increased normalized heart weight by 1 month of age. By 2 months of age there is an increase in fibroblast number, without a significant increase in fibrosis or immune cell abundance (myeloid or T cells). Unlike other models of DCM, D230N mice fail to mount a significant fibrotic response, even at 1 year of age.
Conclusions: Cardiomyocyte hypocontractility induced by D230N expression results in decreased systolic function and an expansion of fibroblasts without significant fibrosis or inflammation by 2 months of age. Ongoing studies are characterizing fibroblast activation and changes in subhistological remodeling of the extracellular matrix.

Title:
Atomistic Modeling of Cardiac Myosin-Binding Protein-C to Elucidate Its Role in Thick Filament Regulation

Presenting Author:
Tristan Wasley, Graduate Student

Addl. Supporting Authors:
Matthew Childers, Postdoctoral Fellow
Mike Regnier, Faculty

Abstract:
Cardiac Myosin-Binding Protein-C (cMyBP-C) is a modulator of cardiac contractility, exerting both inhibitory e ects on the thick filament and activating e ects on the thin filament. While the molecular basis of cMyBP-C's interaction with actin is increasingly well understood, its regulatory role on the thick filament remains less clearly defined. Recent evidence suggests that cMyBP-C may stabilize the super-relaxed state of myosin by sequestering myosin heads to the thick filament backbone through its central domains. Additionally, the N-terminal domains of cMyBP-C have been shown to bind β-cardiac myosin, a process modulated by beta-adrenergic phosphorylation within the intrinsically disordered M-domain. To investigate these interactions at atomic resolution, we constructed a full-length molecular model of cMyBP-C incorporating its disordered linkers and domains. This model was built using a combination of available X-ray, NMR, and AlphaFold models. Improper structural features and clashes were resolved using Chimera and SWISS-MODEL. The model was then prepared using AMBER all-atom molecular dynamics (MD) minimization. It was further developed using microsecond-timescale SIRAH coarse-grained MD simulations, followed by refinement with AMBER all-atom MD simulations with particular focus on the C0, C1, and C2 N-terminal domains, and Proline/Alanine Linker and M disordered regions. Future simulations will incorporate phosphorylation at residues S275, S284, and S311 using the phosaa19SB force field to investigate their e ects on structure and function. The resulting ensembles of phosphorylated and unphosphorylated structures will serve as the basis for flexible docking studies with β-cardiac myosin in its interacting-heads motif conformation, providing mechanistic insight into cMyBP-C–mediated thick filament regulation.

Underlying fibroblast memory regulates fibroblast responsiveness

Darrian Bugg[1,3], Amy Gifford[1], Jennifer Davis[1-4]

Nutrition, Obesity and Atherosclerosis T32HL007028

[1]Department of Lab Medicine and Pathology, [2]Department of Bioengineering, [3]Center for Cardiovascular Biology, [4]Institute for Stem Cell and Regenerative Medicine at the University of Washington

Dynamic fibroblast state transitions underlie heart's fibrotic response but very little is known about the post resolution alterations in fibroblast number and activation state in chronic disease remodeling where the stress is often cyclic. For decades, it was thought that these cells underwent apoptosis during injury resolution, but recent findings suggest that instead, these activated cells may revert to a semi quiescent state. Here genetic lineage tracing is used in combination with an intermittent profibrotic model utilizing stimulation with angiotensin II (Ang) and phenylephrine (PE) to mimic chronic disease. Previously activated fibroblasts transcriptionally return to quiescence weeks after the pro-fibrotic stimulus is removed. After transcriptional regression these once activated cardiac fibroblasts more readily activate when challenged with pro fibrotic agonists in vitro, despite being removed from extracellular cues from the myocardium. The pro-fibrotic functionality of these once activated fibroblasts was muted by knocking out p38 during the first pro fibrotic window in activated Periostin+ fibroblasts. This resulted in significantly less fibrotic remodeling with repeat stimulation suggesting that the original population of fibroblasts that respond and become Periostin+ are the primary arbiters of scar secretion in cyclic disease. These data suggest that there is a key fibroblast population that undergoes activation in chronic disease, and after the disease cues are removed these fibroblasts retain memory of previous activation that makes it easier to respond to repeat stimuli which likely perpetuates fibrotic pathological remodeling in patients with chronic or uncontrolled heart disease.

Title: Investigating the effects of Filamin C deletion on cardiac structure and mechanics

Authors: Zhiyang Xue[1], Liz Johnston[2], Shannon Bosche[3], Christian Mandrycky[3], Kristi Kooiker[4], Tim McMillen[3], Galina Flint[3], Farid Moussavi-Harami[4], Mike Regnier[3,5,6,7], and Joe Powers[1,2,5,6,7]

Affiliations: [1]Mechanical Engineering, [2]Lab Medicine & Pathology, [3]Bioengineering, [4]Cardiology, [5]Center for Cardiovascular Biology, [6]Institute for Stem Cell & Regenerative Medicine, [7]Center for Translational Muscle Research, University of Washington, Seattle WA

Presenting Author: Zhiyang Xue, PhD Student in Mechanical Engineering

Filamin C (FLNC) is a cytoskeletal protein predominantly expressed in skeletal and cardiac muscles, and it interacts primarily with structural proteins in the Z-disk of the sarcomere. Increasing numbers of studies have found that FLNC is associated with different forms of cardiomyopathy, including hypertrophic cardiomyopathy, dilated cardiomyopathy (DCM) and restrictive cardiomyopathy. However, the role of FLNC in cardiomyocytes is still unknown. In our research, we want to test the hypotheses that 1) FLNC helps cardiomyocytes in maintaining cell and sarcomere integrity and 2) FLNC-KO will alter the force transmission, leading to impaired contractile performance and DCM remodeling. To test these hypotheses, we developed a tamoxifen-inducible homozygous and cardiac-specific FLNC-KO mouse model. FLNC protein was eliminated in FLNC-KO mice after two weeks tamoxifen treatment. Echocardiography revealed that by 1-week post-tamoxifen, FLNC-KO mice already exhibit partial left-ventricular dilation and reduced ejection fraction typical of a DCM phenotype. Preliminary skinned trabeculae mechanics experiments suggest that FLNC-KO trabeculae have elevated passive stiffness but no statistically significant changes in maximal force, calcium sensitivity of force, or force redevelopment rate compared with controls. Similarly, isolated myofibril mechanics suggest unchanged maximal force and activation rates, but decreased relaxation rates at sub-maximal calcium for FLNC-KO myofibrils compared with controls. Finally, X-ray diffraction suggested resting FLNC-KO myocytes have increased myofilament lattice spacing and thick filament backbone strain. In future experiments, we will combine our experimental structural and biomechanics analyses with spatially explicit multiscale computational models to further elucidate the role of FLNC in mediating intracellular mechanotransmission in cardiomyocytes.

Title: Bioengineering a rapid-testing platform to assess muscle stem cell fate during regeneration

Authors: Ria Aggarwal, Jason Silver, David Mack
Presenter: Ria Aggarwal, Undergraduate

Abstract

Skeletal muscles, which attach to bone via tendons, are essential for several aspects of human health, including voluntary movement and the regulation of metabolic and homeostatic functions. Skeletal muscles have a high regenerative capacity, restoring tissue function through muscle stem cells (MuSCs). However, despite current knowledge of muscle regeneration, the specific timing and molecular mechanisms that dictate self-renewal fate decisions remain poorly understood. Animals are currently the most comprehensive model of muscle regeneration, incorporating the complex muscle niche and multicellular environment, but raise ethical, economic, and time concerns. Furthermore, existing *in vitro* approaches fail to capture key aspects of MuSC fate. Using engineered muscle tissues (EMT), a muscle organoid with myoblast progenitors, fibroblasts, and extracellular matrix (ECM) initially developed by Dr. David Mack, we aim to create an *in vitro* model that (1) incorporates EdU time-pulse labeling within EMTs to trace MuSC proliferation and self-renewal, and (2) recapitulates key aging phenotypes of MuSC and serves as a robust model to study sarcopenia. We seeded EMTs with iPSC-derived MuSCs and allowed them to self-assemble into contractile organoids, with EdU administered at defined intervals. We assessed muscle force generation and tracked EdU location through immunocytochemistry. Preliminary results demonstrated the technical feasibility of EdU pulse-chase for tracking MuSC fate determination in EMTs. Together, this approach will provide a scalable, rapid-testing platform for muscle-related diseases, thereby enhancing current understanding of muscle regeneration and development of targeted therapies.

Effects of Danicamtiv on human cardiac muscle contraction

Madeleine E. Chastain[2], Amelia Ramirez Vargas[1], Peter O. Awinda[1], Kenneth S. Campbell[3], and Bertrand C.W. Tanner[1]

[1]Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, WA. [2]School of Molecular Biosciences, Washington State University, Pullman, WA. [3]Division of Cardiovascular Medicine, University of Kentucky, Lexington, KY.

Heart failure is a debilitating physiological state where the heart cannot pump enough blood to meet the energetic and metabolic demands of the body. The prevalence of heart failure continues to rise despite new treatments. There has been development of medications to treat heart failure, one of which is called Danicamtiv. Danicamtiv is a myosin activator that targets the myosin cross-bridge cycle, resulting in greater the cardiac muscle force production. We hypothesized there may also be increased myosin head recruitment during the power stroke phase, which could contribute to Danicamtiv increasing cardiac muscle contraction. To test this, we assed the mechanical and contractile properties of human cardiac tissue samples of healthy human donors and patients with heart failure who underwent cardiac transplant. These tissue samples were shipped to us from collaborators at the University of Kentucky. Danicamtiv has been studied in animal models of disease, and is in human clinical trials, though it is not well understood how it directly effects human cardiac tissue function. Our research is intended to determine if the proposed effect of the Danicamtiv changes between healthy donors, and patients with heart failure. Preliminary data suggest that the drug effects cardiac muscle tissue at both the molecular and cellular levels.

2025 CTMR Poster Abstract Theme: Modeling and Metabolism in Muscle Research

Title: Genetically encoded fluorescence lifetime H_2O_2 sensors for quantitative redox imaging

Authors: Yejin Kwon*, Justin Daho Lee*, Yuxuan Wang, Andre Berndt
Presenter: Yejin Kwon (Staff) - interested in giving a lightening talk

Abstract (250-word)

Hydrogen peroxide (H_2O_2) serves as both a key signaling molecule and a source of oxidative stress in aerobic organisms. In cardiomyocytes and skeletal muscle, tightly regulated H_2O_2 dynamics are essential for maintaining excitation-contraction coupling and redox signaling, while excessive accumulation leads to fatigue, contractile dysfunction, and disease progression. Yet, the lack of analytic tools that can precisely visualize specific oxidants in real time has been a major bottleneck in deciphering their physiological roles and the mechanisms underlying redox imbalance. We have previously reported a novel H_2O_2 sensor family, oROS, that enables sensitive monitoring of H_2O_2 in animal models and human stem cell models.

Here, we incorporated fluorescence lifetime modulation, a next-generation fluorescence reporting modality for quantitative imaging. Unlike traditional intensity-based fluorescence reporters, lifetime-based fluorescence reporters enable absolute, quantitative measurement enabling precise cell-to-cell and tissue-to-tissue comparison of oxidative state. We reasoned that fluorescent proteins with higher intrinsic quantum yields, such as mScarlet and mTurquoise, would lead to sensors with large lifetime modulation. Exploration of optimal sensor designs (i.e. fluorescence reporter insertion site and inter-domain linker lengths) was accomplished by a novel microbe-independent cloning pipeline that accelerated generation and screening of sensor variants without bacterial transformation.

The resulting sensors, oROS-R (red) and oROS-C (cyan), exhibited ~0.3-ns fluorescence lifetime shift upon oxidation response to H_2O_2. Together, they provide a powerful platform for multiplexed imaging of redox dynamics, allowing spatiotemporally resolved H_2O_2 signaling in muscle physiology and disease, paving the way for a deeper understanding of redox regulation in health and pathology.

Conformational State of Myosin's Disordered Loop 2 Structure Mediates Actomyosin Association During Crossbridge Formation

Kalen Z. Robeson, Matthew Carter Childers, Kieran Fruebis, Rachelle Soriano, Jennifer Davis, Michael Regnier

Introduction: The formation of the actomyosin crossbridge is crucial for myosin's force generation. While cryo-electron microscopy (cryo-EM) has revealed the actin binding surface of myosin in weakly and strongly bound states, the dynamics of the association and the transition between these states are not well understood. Myosin's disordered loop 2 is believed to mediate actomyosin interactions but is often unresolved in structural studies due to its inherent flexibility.

Objectives: This project aims to investigate the dynamics of myosin's actin-binding regions and precisely define the residues involved in actin binding, particularly focusing on the role of the flexible loop 2 structure. We also aim to explore how disease-causing mutations (E525K and V606M) alter loop 2 conformational state space and its impact on actin binding affinity.

Methods: We employed a combination of molecular dynamics (MD) simulations and large-scale electrostatic calculations. Specifically, we conducted nine 500 ns MD simulations of pre-powerstroke human β-myosin S1, with three replicates each of wildtype and two mutant (E525K and V606M) myosin molecules. This dataset allowed for an in-depth analysis of the conformational state space occupied by loop 2.

Results: Our findings indicate that the movement of myosin's flexible loop 2 structure between activated and inactivated conformations significantly regulates myosin's interaction with actin. Both the E525K and V606M mutations altered loop 2 coiling, leading to enhanced actin binding affinity.

Conclusion: This study highlights the critical role of positive charges on loop 2 for actomyosin interactions. It demonstrates how disease-causing mutations, even without directly altering these charges, can still modify their presentation, thereby impacting actin binding affinity. This work also lends mechanistic understating to our work studying these cells in stem cell derived cardiomyocytes.

Characterization of sarcomeric protein mutations related to the development of cardiac hypertrophy and cellular disarray in transgenic mice.

Taylor Christopherson[1], Kyrah Turner[2], Bertrand C.W. Tanner[1]

[1]Department of Integrative Physiology and Neuroscience; [2]School of Molecular Biosciences, Washington State University, Pullman WA. 99164

Cardiac disease is the leading cause of death in the United States, accounting for every 1 in 5 deaths. Hypertrophic cardiomyopathy (HCM), is the most common genetic cause of cardiac disease. Symptoms of HCM include ventricular wall fibrosis and hypertrophy, impacting the heart's ability to pump blood. A main cause of HCM are mutations of sarcomeric proteins; the structural components of the heart muscle needed to regulate contraction and relaxation. Clinically, in HCM, the most commonly mutated protein is cardiac myosin-binding protein-C (cMyBP-C). However, another mutated protein related to HCM development is cardiac troponin-I (cTnI). Both of these proteins are phosphorylated by the same kinase, protein kinase A (PKA); which results in structural and functional changes for both proteins. This project explores individual and phosphorylation-dependent contributions of cMyBP-C and cTnI. Two transgenic mouse lines for this study were utilized: a cMyBP-C knockout model (cMyBP-C KO), where the cMyBP-C gene is absent, and a non-phosphorylatable cTnI model (cTnI S2A), replacing serine phosphorylation sites (ser23/24) with alanines. We hypothesize that cardiac tissue from the cTnI S2A model will exhibit marginal hypertrophy and disarray, while cardiac tissue from the cMyBP-C KO model will exhibit high levels of hypertrophy and disarray. To assess this, histological preparations and biochemical assays were used to characterize tissue morphology of the cTnI S2A mouse line and contrast it with a wild-type control line and cMyBP-C KO line. Understanding the effects of cMyBP-C and cTnI phosphorylation on tissue morphology can help to dissect molecular underpinnings of diseased cardiac phenotypes.

Penicillin/Streptomycin decreases force output of HiPSC derived cardiomyocytes

Anthony Asencio
Kristi Kooiker
Tim McMillen
Andrew Wescott
Michael Regnier
Farid Moussavi-Harami

Human Induced Pluripotent Stem Cells (hiPSC) can be differentiated into cardiomyocytes and matured for functional assays interrogating contractile health as a function of pharmacologic or genetic perturbation. Protocols use penicillin/streptomycin (P/S) to protect against bacterial contamination in long term culture. However, P/S decreases contractile output. To quantify this effect, we cultured hiPSC (WTC11) derived cardiomyocytes (hiPSC-CMs) with and without antibiotic.

Cells were cultured without P/S until day 21 post differentiation. Cells were replated into either tissue culture dishes, onto compliant patterns, or into Engineered Heart Tissues (EHTs), in the presence or absence of 1X P/S for 14 days. The 2-D culture was assayed with Cal-Brite590. The force and kinetics of activation and relaxation were measured at pCa 5.7 and 4 in myofibrils purified from the cells on compliant patterns.

EHTs cultured without P/S generated higher peak twitch force compared to those cultured in P/S (1.88 ±0.3 vs. 0.64 ±0.1 mN/mm^2), a 65.9% decrease. Time to peak force was slower with P/S (246 ±6.1 vs. 232 ±9.3 ms) and relaxation was not significantly different. Myofibrils cultured in P/S generated significantly lower force (pCa 5.7, 96% decrease and pCa 4, 94% decrease). Interestingly, 2-D culture suggests faster calcium rise with P/S (142.4 vs. 177.7ms), higher peak (48.8%), and slower time to 90% relaxation (410.3 vs. 398.8ms).

In our other experiments, force loss due to P/S decreased our signal-noise ratio, which masked the effects of a small molecule myosin modulator on kinetics and force. These findings suggest caution when using penicillin/streptomycin.

Computational modeling of subcellular structural remodeling results in limited impact on contractile function

Åshild Telle, Patrick M. Boyle, Joseph D. Powers

Background

Many cardiomyopathies alter myocyte structure, stiffness, and force-generation properties through molecular and structural remodeling of the cytoskeleton and sarcomeres. These changes impair cellular mechanics and reduce the heart's ability to generate coordinated contractions and maintain normal pump function. In contrast to animal and *in vitro* models, computational modeling allows these factors to be varied independently, enabling systematic investigation of the individual and combined impact of each.

Methods

We developed a novel spatially detailed computational model of a single cardiomyocyte with explicit representation of connected and unconnected subcellular sarcomere structures. Myocyte morphology and functional parameters were chosen to reflect characteristics observed in mouse models of cardiomyopathies. Spatial structure, material properties, and active tension generation were varied systematically to assess their relative and combined effects on cell deformation and contractile behavior.

Results

Changes in active tension and fold changes in sarcomere stiffness produced proportional effects on relative shortening, whereas alterations in spatial structure had an unexpectedly negligible influence. During contraction, myocytes straightened, exhibiting reduced local buckling and more uniform shortening.

Conclusions & Future Directions

Relative shortening appears robust to structural changes, possibly due to the initially high spatial integrity of the myocyte, which enables multiple parallel pathways for force transmission and mechanical cooperativity. As a next step, coupling the model to myofilament dynamics could provide deeper insight into mechanisms of contractile force loss, while experimental data could be used to quantify relative stiffening.

Identification of structural communication in skeletal Troponin C from molecular dynamics simulations

Matthew Carter Childers[1] and Michael Regnier[1]

[1]*Department of Bioengineering, University of Washington, Seattle, WA 98105*

Dynamic motions are often requisite for proteins to carry out their functions or for their 'activation/deactivation'. Proteins can respond to structural and/or bio-physio-chemical signals by sampling various conformational states in a coordinated fashion. However, the detection of structural communication pathways along which allosteric changes operate eludes techniques that rely on data averaged over time and space. Here we analyze an aggregate 71.9 μs of molecular dynamics simulation data for a 'minimally allosteric' protein, the N-terminal lobe of chicken skeletal Troponin C (N-skTnC), with two major conformational states: binding of calcium ions to apo N-skTnC results in an allosteric conformational change required for N-skTnC function. Using long-timescale simulations we also directly simulate the calcium-driven conformational conversion. We have developed a novel algorithm for a global analysis of the structural communication pathways along which allosteric signals are transmitted. Our algorithm is built on a combination of local mobility analysis, dynamic correlated motions analysis, principal component analysis, and information-based graph theory and identifies meaningful relationships within N-skTnC as well as between N-skTnC and its environment. Importantly, we perform a novel sensitivity analysis for each step in our algorithm to identify which adjustable parameters have the greatest influence over the observed measures of allostery. Finally, we explicitly introduce two adjustable parameters that enable allosteric analysis to be scaled across different temporal and spatial scales. We use this analysis to map out critical sets of communicating residues within a given protein fold and make predictions on the effects of structural perturbations to the system.

Characterization of Electrophysiological and Mechanical Properties of MEDUSA Cardiomyocytes for Cardiac Remuscularization Therapy

Noah Jackson Bowers[1], Silvia Marchiano[2]
[1]Department of Bioengineering University of Washington
[2]Department of Pathology, University of Washington
[1,2]Institute of Stem Cell Research and Regenerative Medicine

Abstract

When patients experience heart damage, a downward spiral of increased heart strain and adverse cardiac events occurs, leading to death. Using stem cell therapy, we can reverse this heart damage and restore the integrity of the heart. Ordinary stem cell derived heart muscle cells induce dangerous arrhythmias when directly grafted onto heart tissue, so we developed a novel line of non-spontaneously beating stem cell derived heart muscle cells for use in treatment, designated MEDUSA (Modification of Electrophysiologic DNA to Understand and Suppress Arrhythmia). This cell line carries gene edits altering currents of Na^+, K^+, and Ca^{2+} ion flow within the cell, preventing them from spontaneously depolarizing and creating engraftment arrhythmias. The goal of my project is to characterize the phenotype of the MEDUSA cell line to assess its future and development in cardiac remuscularization therapy. I characterized the MEDUSA maturation state, electrical state, and mechanical state by employing immunofluorescence, fluorescence based calcium recording, and engineered heart tissues respectively. Early immunofluorescence data support the conclusion that MEDUSA possesses an immature mechanical protein expression profile. Furthermore, altered ion channel proteins lead to slow extrusion of calcium from the cell, affecting the kinetics of the MEDUSA action potential. Once analyzed, the artificial heart muscle tissues I've cast will inform the contractile implications of MEDUSA's altered developmental and electrical state. This work will inform the future development of this cell line and in the development of all stem cell derived heart muscle grafts, supporting the future of regenerative therapies in the field of medicine.

Title: Assessing myocyte–targeted reversibility of inherited dilated cardiomyopathy

Authors: Isabella M Reichardt, Abby Nagle, Kristina Kooiker, Elizabeth Plaster, Jagadambika Gunaje, Sasha Smolgovsky, Elaheh Karbassi, Rachelle Soriano, Michael Regnier, Farid Moussavi-Harami, Jennifer Davis

Dilated cardiomyopathy (DCM) is one of the most common inherited cardiac diseases, characterized by systolic dysfunction, ventricular dilation, myocyte hypertrophy, and fibrosis. Myofilament activators have recently emerged as a new class of targeted therapies to treat DCM; however, preliminary clinical trials have produced lukewarm results. To assess the potential of myocyte-targeted therapies to reverse DCM, we utilized a tet-off mouse model that expresses a DCM-inducing mutant cTnC (I61Q) that can be silenced using doxycycline (DOX). Administration of DOX normalized Ca^{2+} sensitivity within 7 days, indicating the I61Q transgene turns off rapidly with DOX. I61Q mice were aged out to six months and then given one month of DOX to assess the capacity for reversal after disease onset. I61Q myocyte hypertrophy was normalized to wildtype levels and whole heart ejection fraction and dilation were partially rescued. However, fibrotic deposition and increased fibroblast number was not reversible. To determine if there is a point at which the heart cannot recover, I61Q mice were aged to the point of significant mortality and then given one month of DOX. Even at the stage of severe disease, there were remarkable improvements in myocyte hypertrophy, whole heart ejection fraction, and ventricular dilation. Similar to earlier timepoints, fibrotic remodeling was not reversible. These results indicate that myocyte-targeted treatments are incredibly effective at abrogating myocyte remodeling and improving whole organ function even after significant maladaptive remodeling has occurred. However, the persistence of fibrotic remodeling suggest that combinational therapies may be necessary to achieve complete disease reversal in DCM.

What mechanisms can explain faster force-time rise when using pre-activating vs. relaxing solutions?

Sadra Amirabadi[1], Austin K. Au[2], David C. Lin[1,2], Bertrand C.W. Tanner[2]

[1]Voiland School of Chemical Engineering and Bioengineering, and [2]Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, WA.

In skinned skeletal muscle fibers, force develops faster when fibers are initially incubated in low-[Ca^{2+}] pre-activating (PA) solution prior to being transferred to high-[Ca^{2+}] activating (A) solution, versus initial incubation in relaxing (R) solution. PA contains less EGTA than R; EGTA is a Ca^{2+} chelator that binds free Ca^{2+} to form CaEGTA. To test whether CaEGTA buffering alone can explain the faster force rise, we combined single-fiber experiments with computational models predicting activation dynamics and force production. Single rat type IIb fibers were switched from either PA→A or R→A solutions, and isometric force was recorded. Half-rise time (time to reach 50% of maximal force) was substantially shorter for PA→A (0.43±0.14 s) than R→A (2.61±0.63 s). We then modeled radial Ca^{2+} diffusion and reversible CaEGTA binding in a cylindrical fiber to predict intracellular Ca^{2+} transients for either PA→A or R→A. The resulting Ca^{2+} transients were used as inputs for FiberSim, a spatially explicit half-sarcomere contraction model. We explored broad parameter ranges within FiberSim and only kept simulation results that were consistent with physiological estimates of myofilament activation levels and experimental R→A force dynamics. The diffusion model supported reduced Ca^{2+} buffering in PA as a contributor to faster force rise. However, the simulations did not reproduce the dramatically faster force rise with the simulated PA→A activation (1.72±0.62 s for PA→A vs. 2.43±0.35 s for R→A). This suggests rapid Ca^{2+} delivery is necessary but insufficient to explain the rapid force development observed in the experiments. Future modifications could model Ca^{2+} sensitivity of cross-bridge.

SPATIAL DISTRIBUTION OF ADHESION FORCE GENERATION IN HUMAN CARDIOMYOCYTES

Nagle A, Leung C, Regnier M, and Davis J

Cardiomyocytes (CMs) sense macro scale mechanical cues through their focal adhesion (FA) connection to the extracellular matrix (ECM) to regulate cell structure and function. However the molecular mechanisms by which CMs sense tensile force at FAs during the cardiac cycle are still unclear. Current understanding of cell adhesion force in CMs relies on measurements of whole cell contractile tension or traction stress. FAs of other cell types have been shown to distribute FA tension unevenly within individual cells and adhesions. Furthermore, in CMs there are regional heterogeneities in contractile strain. Therefore it is important to understand the spatial distribution of FA tension with respect to CM contraction to determine the fundamental degree and distribution of mechanosensing in the cell as the myofibrils contracts against the ECM. To determine FA tension generation during CM contraction, we have utilized the first endogenously expressed FRET tension sensor in induced pluripotent stem cell (iPSC) derived CMs to examine the static and dynamic tension generation due to contraction. This new model allows for measurement of the key forces sensed at the FA complexes, specifically the protein vinculin, while overcoming the challenges of inconsistent sensor expression or artifacts from vinculin overexpression that may interfere with proper data acquisition. We utilize a novel method for examining static contraction of CMs that eliminates artifacts from temporal movement and reduces image acquisition complexity. Our data shows myofibril contraction confers an increase in global FA tension sensation in static CMs, and that there are differential distributions of both adhesion force and sarcomere placement in relaxed vs contracted cells. Ongoing studies are examining spatial and temporal generation of FA force in CMs during contraction in live cells, as well as the relationship between CM passive tension and FA tension.

The G256E HCM mutation prolongs relaxation via altered nucleotide handling

Kerry Y. Kao[1,2], Matthew Carter Childers[1,2], Divya Pathak[3], Rama Reddy Goluguri[3], Timothy S. McMillen[4], Kathleen M. Ruppel[3], James A. Spudich[3], and Michael Regnier[1,2]

[1]Department of Bioengineering, University of Washington, Seattle, WA 98105
[2]Center for Translational Muscle Research, University of Washington, Seattle, WA 98105
[3]Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305
[4]Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA 98109

Genetic mutations in sarcomeric proteins are linked with familial forms of hypertrophic cardiomyopathy (HCM). Human induced pluripotent stem cell-derived cardiomyocytes have emerged as a powerful system to study the effects of HCM-linked mutations on impaired cardiac performance. Combined with high-resolution structural models of actin and myosin, these systems enable integrated structure–function analyses of sarcomeric variants. Using an interdisciplinary approach, we analyzed the myosin variant G256E at multiple scales. At the atomic and molecular scale, molecular dynamics (MD) simulations revealed that G256E induces structural changes in the nucleotide-binding pocket, indicative of an increased affinity of myosin for ADP, which was further validated through stopped-flow biochemistry. Steered MD simulations suggested alterations in the timing and sequence of contact pair disruptions and an increase in the work required to displace ADP·Mg^{2+} from the binding pocket. In myofibrils, delayed ADP release manifested as a prolongation and reduced ADP sensitivity of the early phase of relaxation, believed to represent the rate of cross-bridge detachment. These findings highlight the importance of ADP release in the chemo-mechanical cycle and identify the molecular mechanisms by which an HCM-linked mutation leads to disease phenotypes by modifying ADP release. Furthermore, this study demonstrates that small-scale changes in the structure of myosin's active site can lead to kinetic changes in actomyosin cycling that manifest as elevated force generation and impaired relaxation through large-scale changes in cross-bridge cycling. This work demonstrates how integrating computational, biochemical, and cellular models can elucidate both the phenotypic and biophysical origins of HCM.

Phosphorylation-Dependent Coordination of RLC and cMyBP-C Regulates Sarcomeric Dynamics in Hypertrophic Cardiomyopathy

Adriana Billante[1], Tristan Wasley[2,3], Matthew Childers[2,3], Michael Regnier[2,3], Bertrand Tanner[4]

[1]School of Molecular Biosciences, Washington State University, Pullman, Washington. [2]Department of Bioengineering, College of Engineering & UW Medicine, University of Washington, Seattle, Washington. [3]Center for Translational Muscle Research, University of Washington, Seattle, Washington. [4]Department of Integrative Physiology & Neuroscience, Washinton State University, Pullman, Washington.

Hypertrophic cardiomyopathy (HCM) is a leading cause of sudden cardiac death in young adults, yet its molecular basis remains poorly defined. This disease is characterized by left ventricular wall thickening and hypercontractility arising from dysregulated actin–myosin cross-bridge interactions. Sarcomeric performance depends on a coordinated network of regulatory proteins that govern both contraction and relaxation. Among these are myosin regulatory light chain (RLC) and cardiac myosin-binding protein C (cMyBP-C), which play complementary roles in tuning thick-filament regulatory activity and contractile dynamics. RLC stabilizes the myosin lever arm and modulates the proximity of myosin heads to actin, while cMyBP-C interacts with both myosin and actin through its N-terminal domains to regulate cross-bridge recruitment.

Mutations in *MYBPC3*, which encodes cMyBP-C, are among the most common causes of familial HCM, emphasizing the importance of precise regulatory control in maintaining normal cardiac function. Despite this, the structural and mechanistic basis of how cMyBP-C and RLC coordinate thick-filament dynamics remains unresolved. Post-translational phosphorylation of these proteins adds an additional layer of complexity to their regulatory activity; RLC phosphorylation is thought to accelerate cross-bridge cycling and enhance force generation, whereas cMyBP-C phosphorylation likely promotes relaxation by reducing myosin binding. Disruption of this phosphoregulatory balance is believed to destabilize contractile dynamics and contribute to HCM pathogenesis.

This study aims to elucidate how phosphorylation of RLC and cMyBP-C cooperatively modulates sarcomeric mechanics. Using truncated recombinant constructs and biochemical binding assays in both phosphorylated and unphosphorylated states, we aim to map domain-specific interactions to clarify how phosphorylation-dependent regulation influences thick-filament function in health and disease.

High Throughput Pooled Gain-of-Function Screen Reveals Regulators of Human Stem Cell-Derived Cardiomyocyte Maturation

Elaheh Karbassi, Xiulan Yang, Silvia Marchiano, Shin Kadota, Silvia Domcke, Hans Reinecke, Jordan Klaiman, Charles E Murry

Human pluripotent stem cell-derived cardiomyocyte (hPSC-CM) applications for cell therapy and disease modeling are limited due to the cells' lack of resemblance structurally and functionally to their adult counterparts. Unlike adult cardiomyocytes, hPSC-CMs are small in size, generate low contractile force, rely primarily on glucose as their energy source (rather than fatty acids) and have pacemaking properties. To understand hPSC-CM maturation, we characterized two established approaches to mature cardiomyocytes—long term culture (aging of cells in a dish) and *in vivo* transplantation to an infarcted adult rat heart. RNA sequencing of hPSC-CMs from these systems demonstrated that *in vivo* transplantation is much more effective in maturing hPSC-CMs, strongly inducing a more adult-like cardiac gene program (e.g. upregulation of *TNNI3*, *MYL2*, *SCN5A*), compared to cells kept in culture up to 1 year. Using this dataset, we identified candidate drivers of hPSC-CM maturation, including transcription factors and chromatin regulators, that we hypothesize are necessary to program hPSC-CMs to an adult-like state. We performed a high throughput pooled CRISPR activation screen, with a single cell RNA sequencing readout, to activate and test the role of 108 candidate genes in hPSC-CM biology and maturation. We identified subsets of transcriptional regulators, when activated, are associated with and shift cells towards a more mature cardiac state. Ongoing work aims to validate these factors and identify key combinations that are effective in transcriptionally programming hPSC-CM maturation for cardiac regeneration.

Role of skeletal muscle AMPKα2 in chemotherapy-induced cachexia

Haiming L. Kerr, Nornubari Myree, Elizabeth Dacek, Kora Krumm, Anthony Christiani, Jessica Li, Suah Kim, Jose M. Garcia

Gerontology and Geriatric Medicine, University of Washington Department of Medicine, Seattle, WA 98195, USA; Geriatric Research, Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA.

Cisplatin is a widely used chemotherapeutic agent, but its clinical benefits are often accompanied by cachexia-related symptoms. Effective interventions to mitigate these adverse effects remain limited. 5'-adenosine monophosphate-activated protein kinase (AMPK), an intracellular energy sensor typically activated by exercise and decreased food intake. This study aimed to investigate the role of AMPK in chemotherapy-induced muscle wasting and its potential role as a therapeutic target. Adult male wild-type (WT) and muscle-specific AMPKα2-inactive transgenic (AMPKα2i TG) FVB mice received daily cisplatin or saline injections for 5 days, followed by a 4-day recovery before terminal analyses. Results: At baseline, TG mice showed impaired endurance and greater fatigue, with a reduced proportion of slow-twitch fibers compared to WT. Cisplatin reduced food intake, body weight, fat mass, and lean mass in both genotypes, although fat loss was less pronounced in TG mice. Muscle mass was modestly reduced, and endurance was unaffected by cisplatin in either genotype. Notably, cisplatin impaired grip strength in TG but not WT mice. Basal fatty acid oxidation (pACC/ACC ratio) was lower in TG than WT, but cisplatin elevated pACC/ACC in TG mice to WT levels. TG mice also exhibited consistently higher autophagy markers (LC3II/I) and lower mitochondrial biogenesis marker (PGC-1α) expression than WT, independent of cisplatin. Conclusions: AMPK is critical for endurance and fatigue resistance by maintaining mitochondrial biogenesis and slow-twitch fiber function. Loss of AMPKα2 activity selectively impaired muscle strength but attenuated fat loss, indicating its context-dependent role in chemotherapy-induced cachexia.