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Abstract Book

Poster Presentations

 **Indicates the presenter is also giving a lightning talk.**

Ablation of cardiac myosin binding protein-C induces cell signaling for fibrosis and hypertrophy prior to morphological remodeling of the myocardium

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

[1]School of Molecular Biosciences & [2]Dept. of Integrative Physiology & Neuroscience, Washington State University, Pullman, Washington

Hypertrophic cardiomyopathy (HCM) is the leading genetic cause of heart disease. Although research has been focused on this condition for the last several decades, clinical treatments for patients with HCM remain limited. Current therapies focus on decreasing cardiac stress rather than treating the underlying genetic condition. The heart comprises several myofilament proteins that work together to facilitate proper contraction and relaxation to pump blood throughout the body. Mutations in cardiac myosin binding protein-C (cMyBP-C) are frequently linked with clinical cases of HCM. To further understand the role of cMyBP-C and its contribution to cardiac disease, we assessed the progressive development of morphological and molecular biomarkers associated with HCM in a transgenic mouse model that lacks cMyBP-C. We assessed gene expression associated with sarcomeric proteins and HCM development via a custom cardiac gene panel using Nanostring nCounter analysis. Morphological alterations in cardiac tissue were evaluated using biochemical and histological assays. Our findings unveil significant dysregulation in genes associated with fibrosis and hypertrophy in cMyBP-C deficient mice as early as 21 days after birth, preceding observable morphological changes in the cardiac tissue. Alterations in expression of sarcomere specific proteins begin after notable cardiac remodeling. The early alterations to gene expression underscore the need for better understanding the mechanisms driving HCM development, which may offer avenues for therapeutic intervention before pathological remodeling occurs. Pharmaceutical interventions that target cardiac dysfunction are most viable prior to substantial cardiac remodeling, highlighting the potential utility for early screening and preventative strategies to manage genetic-based cardiomyopathies.

Circumferential Strain Recovery after Human Cardiomyocyte Transplantation in Minipigs using a Novel Frequency-based Method for Myocardial Tagging and Quantification

Anna V. Naumova[1], Kenta Nakamura[2,3], Silvia Marchiano[2,4,5], Lauren E. Neidig[2,4,5], Leslie P. Blakely[2,4,5,6], Hiroshi Tsuchida[2,4,5,6,7], Charles E. Murry * [2,4,5,8,] William S. Kerwin * [1,9].

[1] Department of Radiology, University of Washington, Seattle, WA, USA.
[2] Institute for Stem Cells and Regenerative Medicine, University of Washington, Seattle, WA, USA.
[3] Department of Medicine, Division of Cardiology, University of Washington, Seattle, WA, USA.
[4] Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA, USA.
[5] Center of Cardiovascular Biology, University of Washington, Seattle, WA, USA.
[6] Department of Comparative Medicine, University of Washington, Seattle, WA, USA.
[7] Currently at Fred Hutchinson Cancer Center, Seattle, WA, USA.
[8] Currently at Department of Stem Cell Biology and Regenerative Medicine, University of South California, Los Angeles, CA, USA.
[9] Currently at Altius Institute for Biomedical Sciences, Seattle, WA, USA.
* Co-senior authors.

Background. Transplantation of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) is promising new method for heart remuscularization after infarction. We hypothesized that hPSC-CMs affect heart recovery by improving local contractility in the infarcted zones. A precise non-invasive assessment of regional contractile function in the infarcted segments of the heart is required.

Methods. We describe a novel approach for rapid and robust quantification of myocardial end-systolic circumferential strain (CS). Linear tags are placed in 60-degree pattern offsets and analyzed via optimized post-processing based on local Fourier transformation of the defined American Heart Association segments. This method has been implemented for the first time to evaluate transendocardial hPSC-CMs transplantation in a minipig model of myocardial infarction.

Results. In the cell-treated hearts (n=4), mean end-systolic CS in the infarcted segments at the mid-wall region decreased from -6.69 ± 1.56% (pre-MI) to -1.13 ± 1.96% at 2 weeks post-MI (pre-treatment), with subsequent improvement to -4.00 ± 0.76% by 8 weeks after cell transplantation. Conversely, CS in the infarcted segments in vehicle-control group (n=5) decreased from -5.18 ± 0.97% (pre-MI) to -1.39 ± 1.23% at 2 weeks post-MI and worsened further to 0.33 ± 1.93% by 8 weeks. There was no improvement in the global ejection fraction in the cell-treated group in comparison with control.

Conclusions. A novel technique for assessment of local circumferential strain is a more sensitive measure of regional myocardial dysfunction than global contractility. Our findings confirm our hypothesis that transplantation of hPSC-CMs improves regional myocardial strain in the infarcted zone of the minipig's heart.

Mechanical modeling of cardiac fibrosis with explicit spatial representation of myocyte loss and collagen alignment

Åshild Telle[1], Mary M. Maleckar[2], Samuel T. Wall[2],
Christoph M. Augustin[3], Joakim Sundnes[2], Patrick M. Boyle[1,4]

[1]Department of Bioengineering, University of Washington, Seattle, USA
[2]Department of Computational Physiology, Simula Research Laboratory, Oslo, Norway
[3] Medical University of Graz, Graz, Austria
[4]Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, USA

Abstract

Cardiac fibrosis, a pathological condition associated with various diseases, alters tissue mechanics and reduces cardiac contractility. Common forms include replacement fibrosis, where damaged myocytes are replaced by collagen, and interstitial fibrosis, characterized by matrix expansion between myocytes. This happens simultaneously with other remodeling mechanisms, such as tissue stiffening and collagen alignment. The implications of the individual mechanisms taking place remain poorly understood.

In this work we investigated microscale mechanical effects of fibrotic remodeling using a computational model that explicitly represents myocyte and collagen structures. We simulated replacement fibrosis by removing 50% of the myocytes, and interstitial fibrosis by increasing gaps between the myocytes. Next, we assessed the impacts of increased stiffness and collagen alignment on both configurations through contraction and stretch experiments.

Our findings reveal substantial contractility impairment in replacement fibrosis, and highly heterogeneous stress distributions. Collagen alignment in the myofiber direction helped mitigate high stress concentrations. Interstitial fibrosis had minimal impact on strain and stress patterns. However, interstitial fibrosis combined with increased myocyte and matrix stiffness and aligned collagen led to the highest increase in tissue-level stiffness values.

In summary, replacement fibrosis primarily affects contractility, while interstitial fibrosis mainly influences tissue stiffness. Collagen alignment appears to be a key compensatory factor that reduces stress in myocyte loss and helps restore cardiac anisotropy. Future work integrating modeling with experimental data could provide deeper insights into cardiac fibrosis mechanisms.

Metabolomic Perturbations during Androgen Deprivation Therapy are Associated with Decline in Function but not Lean Mass

Lindsey J. Anderson[1,2], Atreya Dash[3,4], and Jose M. Garcia[1,2]

[1]Geriatric Research, Education and Clinical Center, [3]Department of Urology; Veterans Affairs Puget Sound Health Care System, Seattle, Washington

[2]Division of Gerontology and Geriatric Medicine-Department of Medicine, [4]Department of Urology; University of Washington, Seattle, Washington

Background: Androgen deprivation therapy (ADT) is the standard treatment for advanced/ metastatic prostate cancer (PCa) but ADT-induced sarcopenia (muscle wasting/functional decline) worsens quality of life and survival. We hypothesized that metabolomic perturbations would be associated with ADT-induced sarcopenia.

Methods: In men with PCa, we assessed **appendicular lean mass (ALM), performance** (handgrip strength, stair climbing power “SCP”, 6-minute walk test “6MWT”, aerobic capacity); **quadriceps and plasma targeted metabolomics**; and **patient-reported outcomes** (EORTC-QLQC30) before and six months after initial ADT. Paired t-tests (between time-points) and spearman correlations (change associations) were used ($p \leq 0.05$). Metabolanalyst6.0 analyzed metabolomic data with Benjamini-Hochberg adjustment (FDR<0.1).

Results: Outcomes were evaluated in patients with paired plasma (n=25) and muscle (n=10/25) metabolomics. After 6-months, ALM, performance, and EORTC-QLQC30 Physical Function and Fatigue worsened ($p \leq 0.027$); 6MWT trend ($p=0.078$). No plasma pathways were altered. Six plasma metabolites were significantly altered (FDR>1) including guanidinoacetate and 5-aminovaleric acid which are associated with arginine/proline metabolism (pathway analysis: $p=0.07$). 5-aminovaleric acid increases correlated with worsened 6MWT and QLQ-Physical Function ($r=-0.48$- -0.57, $p \leq 0.02$). In quadriceps, beta-Alanine metabolism and alanine/aspartate/glutamate metabolism displayed a trend ($p<0.1$, FDR>1). Five nominally altered metabolites (N-acetyl-aspartate, aspartic acid, asparagine, pantothenate, glutamine; $p \leq 0.05$) are associated with these two pathways. N-acetyl-aspartate increases were correlated with QLQ-Fatigue increases ($r=0.69$, $p=0.04$). Glutamine decreases were correlated with SCP decreases ($r=0.70$, $p=0.04$). No pathways/metabolites were associated with ALM.

Conclusion: Metabolomic perturbations were associated with worsened performance and patient-reported outcomes, but not ALM, suggesting that these pathways are androgen-responsive and may represent therapeutic targets for mitigating functional decline during initial ADT.

Conditions for Optimal Acute Retention of Stem Cell-derived Cardiomyocytes for Intramyocardial Injections in Sprague Dawley Rats

Trevor Mollot[1,2], Leslie Blakeley[1], Michael Malone[2,3], Kenta Nakamura[1,2,4,5], Charles E. Murry[1,2,4], Nathan J. Sniadecki[1-3]

[1]Bioengineering Department, University of Washington

[2]Intitute for Stem Cell and Regenerative Medicine, University of Washington

[3]Mechanical Engineering Department, University of Washington

[4]Cardiology Department, University of Washington

[5]UW Center for Cardiovascular Biology

Cell therapies for heart regeneration using pluripotent stem cell-derived cardiomyocytes (PSC-CMs) have shown improvements in restoring heart function. However, the efficacy of current approaches is limited by poor retention of cells injected into the myocardium. In this work, we investigated the effects of injection volume and number of injection sites of PSC-CMs in a rodent model. Results to date show that 3 injections comprising the same overall volume as a single injection of cell suspension offer more consistent, and overall higher retention in the acute phase. However, when injection number is increased to 9, overall retention drops below that of a single large bolus. This may be elucidating two mechanisms of loss: one due to pressure-driven expulsion from the large cell bolus itself, and one due to the excessive puncturing of the heart tissue, leading to leakage out of the needle tracks during normal cardiac contraction. Current research endeavors are aiming to further characterize these mechanisms of acute loss. We predict there exists an optimal ratio of injection number and volume for maximal retention in any heart model, speaking to the translatability of this work.

A direct reprogramming of fibroblast into skeletal muscles targeting receptor signaling pathways with AI-designed heterofusions

Riya Keshri[1,2], Zachary Foreman[2,8], Damien Detraux[1,2], Ashish Phal[1,2], Marc Exposit[5], Mohamad Abedi[5], Shruti Jain[5], Tung Ching Chan[2], Yen-chian Lim[2], Sanjay Srivatsan[3,4], Beatriz Estrada[2,7], Alec ST Smith[2,6], David L Mack[2,5,6], Jay Shendure[1,2,3], David Baker[1,4], Julie Mathieu[1,2,5], Hannele Ruohola-Baker[1,2]

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. Department of Genomics, University of Washington, School of Medicine, Seattle, United States. 4. Institute for Protein Design, University of Washington, School of Medicine, Seattle, United States. 5. Department of Comparative Medicine, University of Washington, Seattle, United States. 6. Department of Physiology and Biophysics, University of Washington, Seattle, WA, USA, 7. Institute of Biomedicine of Seville, Spanish National Research Council (CSIC) Seville, Spain. 8. Department of Biology, University of Washington, Seattle, United States.

Abstract

Transdifferentiation, the process of directly converting cells from one somatic cell type to another, has emerged as a promising method for regenerative medicine. However, the process is currently limited by a low efficiency of conversion. Here we show that AI-designed synthetic minibinders for tunable signaling pathways can result in high efficiency direct reprogramming. Our laboratories have previously revealed that synthetic minibinders (mb) show high receptor specificity, unprecedented receptor isoform specificity and better stability than the natural ligands. These synthetic mb as monomers are antagonists for RTK/RSTK signaling, but when scaffolded at high valence, act as agonists. A third category of minibinders, Heterofusions forcefully bind to two normally nonpairing receptors and bring them in close proximity to elicit unprecedented signaling response. We identified designed minibinder combinations, Combo Cocktails (FGFR1/2c or TRkA designed agonist in combination with ALK1R and TGFBRII designed antagonists) that significantly increased the efficiency of myogenic transdifferentiation in human fibroblasts. These data show that concomitant FGFR1/2C-splice variant and Alk1 and TGFbR2 pathway inactivation is critical for the direct reprogramming process. By Bulk sequencing and single cell sequencing analysis we found that expression of myogenic regulators (ID3 SNAI1), HGF, myokine Fibcd1 known to regulate myofiber size are upregulated in the minibinders cocktail treatment. Next, we show some of the designed **heterofusion**s (for example, Her2mb-FGFRmb) enhanced trans differentiation efficiency, even without the combo cocktail. Her2 is a key RTK of EGFR signaling but lacks a known ligand. Here, we show AI designed synthetic agonists combining HER2 and FGFR (HER2mb fused to FGFR1/2c-mb; H2F) elicit potent signaling responses. H2F forcefully binds to two normally non-pairing receptors, HER2 and FGFR bringing them in close proximity to elicit an unprecedented signaling response. HER2 interaction bifurcates FGFR-activity to MAPK pathway eliminating PLCg-Ca2+ signaling and resulting in significant increase in the efficiency of myogenic transdifferentiation in human fibroblasts. H2F agonist induces skeletal transdifferentiation more potently than any previous ligands. We further show that some of these heterofusions also improved the hiPSCs derived myogenic differentiations such as TRKAmb:BMPR2mb. Designed heterofusions will have an enormous impact on regenerative therapeutics advancement.

A molecular scale investigation of the mechanisms of contractile dysfunction for the hypertrophic cardiomyopathy MYH7 G256E mutation

Kerry Y. Kao[1], Matthew C. Childers[1], Tim McMillen[1], Michael Regnier[1]

[1]University of Washington, Department of Bioengineering, Seattle, WA 98195

Studying the molecular mechanisms of hypertrophic cardiomyopathy (HCM) in humans has several challenges, including availability of myectomy samples for specific mutations and history of patient HCM management. To determine the effect of mutation-specific effects on β-myosin heavy chain (MHC) function during contractile behavior, we use CRISPR-Cas9 gene-editing of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as a model system. Measurements from individual isolated contractile organelles (myofibrils) from hiPSC-CMs demonstrate that the specific force and rate of force generation for mutant MYH7 G256E myofibrils is greater than isogenic control myofibrils. During relaxation, the initial, slow phase kinetics were decreased, indicating slower cross-bridge detachment rate. Furthermore, isolated G256E myofibrils were less sensitive to ADP product inhibition during relaxation, pointing to delayed ADP release as a mechanism of impaired relaxation. In simulations of the post-rigor (M.ATP) and rigor state structures, we observed reduced stability in the transducer region, due to weakened hydrogen bonds between neighboring β-sheet strands as well as significant changes in local contacts. In post-powerstroke simulations (A.M.ADP), altered nucleotide pocket dynamics in G256E simulations suggest that structural communication may be affected. Furthermore, adaptive steered MD simulations demonstrated that ADP requires more free energy to dissociate from G256E post-powerstroke myosin, supporting impaired ADP release. Combining our *in silico* and experimental data, our results suggest that the G256E mutation affects the myosin transducer region such that it alters communication between the nucleotide binding pocket and the actin binding surface during the acto-myosin chemo-mechanical cycle. This leads to hypercontractility and slowed relaxation.

MYH3 mutations associated with distal arthrogryposis alter the contractility and myosin isoform switching of hiPSC derived skeletal muscle

Christian Mandrycky[1], Saffie Mohran[1], Matthew Childers[1], Shawn Luttrell[2], Elizabeth Choi[1], Kati Buckingham[3], Michael Bamshad[3], David L. Mack[1,2], Michael Regnier[1]

[1]Department of Bioengineering, [2]Department of Rehabilitation Medicine, [3]Department of Pediatrics, University of Washington, Seattle, Washington

Distal arthrogryposis (DA) is a skeletal muscle disorder characterized by joint contractures predominantly localized in the distal extremities. DA associated syndromes like Freeman-Sheldon Syndrome (FSS) are linked to mutations in the *MYH3* gene that encodes the embryonic skeletal muscle myosin. To study the mutation and its effect on developing muscle, we generated human induced pluripotent stem cell (hiPSC) lines bearing T178I or R672C *MYH3* mutations. hiPSC were differentiated into skeletal muscle and evaluated for differences in the maturation of the contractile unit and its functional performance. All lines efficiently differentiated into skeletal muscle and no differences in fusion efficiency or sarcomere length were observed between genotypes. R672C mutations in MYH3 were associated with alterations in myosin isoform content, with homozygous R672C having reduced MYH3 protein by day 7 of differentiation and near loss of MYH3 protein by day 9. This corresponded with elevated levels of MYH8 and MYH7 protein relative to isogenic control. Myofibril mechanics measurements showed no differences in specific force between mutant and normal myofibrils, but myofibrils from homozygous R672C cells had a faster rate of relaxation ($k_{rel,fast}$) and more complete relaxation. To investigate the functional effect of MYH3 mutations absent changes in myosin isoform content we have validated the siRNA induced knockdown of MYH8 and MYH7 in normal and mutant cells. Ongoing studies will further characterize the effect of these mutations on actin-myosin binding, crossbridge cycling kinetics, the maturation of skeletal myotubes over time, and myosin isoform switching dynamics.

Effect of the E525K myosin mutation on myofilament contraction—understanding hypocontraction in the context of increased ATPase activity

Kalen Z Robeson, Kieran Fruebis, Rachelle Soriano, Jennifer Davis PhD, Michael Regnier PhD

The *de novo* myosin mutation E525K was identified in 2012 in a single patient diagnosed with dilated cardiomyopathy (DCM). Recent work on isolated engineered myosin constructs has led to the hypothesis that this mutation stabilizes the interacting heads motif (IHM) and increases the intrinsic rate of the myosin ATPase—the overall effect being hypocontractile due to impaired interaction with actin. However, to date no measurements have been made in myofilaments or cardiomyocytes to understand the effect of this mutation on force generation *in situ*. Here we present the first force and contractility measurements generated from induced pluripotent stem cell (iPSC) derived cardiomyocytes (CMs) engineered to carry the heterozygous E525K myosin mutation. Live cell imaging of sarcomere contraction demonstrated decreased sarcomere shortening and decreased sarcomere organization. Conversely, mechanics measurements from isolated myofibrils stimulated with saturating calcium (pCa 4.0) demonstrated increased maximum force (Fmax) and an increased rate of force development (Kact). These disparate results may be driven by altered calcium sensitivity, which we will determine by measuring force and contractile kinetics of myofibrils with submaximal calcium activations. Our findings underscore the importance of investigating myosin mutations using both biochemical and biophysical approaches. This research also underscores the importance of understanding whether disease phenotypes are caused by alterations in myosin recruitment or changes in the myosin chemo-mechanical cycle, or both, in thinking about potential novel myosin targeted therapeutic strategies.

Molecular dynamics models demonstrate alterations in troponin's function caused by cardiomyopathy associated mutations

Sage Malingen[*1,2]; Matthew Carter Childers[*1,2]; Travis Tune [2,3]; Kerry Kao [1,2]; Thomas Daniel [2,3]; Farid Moussavi-Harami [2,4,5]; Michael Regnier [1,2]

*co-first authors

1. Department of Bioengineering, University of Washington, Seattle, WA 98109
2. Center for Translational Muscle Research, University of Washington, Seattle, WA 98109
3. Department of Biology, University of Washington, Seattle, WA 981053.
4. Department of Laboratory Medicine & Pathology, University of Washington, Seattle, WA 98105
5. Division of Cardiology, University of Washington, Seattle, WA 98109

The sarcomeric protein troponin regulates the timing of muscle contraction by undergoing a dramatic shape change following calcium binding. This shape change ultimately allows tropomyosin to move away from prospective binding sites along the thin filament and myosin motors to bind to the thin filament. Mutations in troponin can disrupt its function, and several are implicated in cardiomyopathies. In this study, we explore how mutations within TnC alter its structure and dynamics using molecular dynamics simulations. We have performed these studies with all three troponin subunits, allowing the mutation's effects to propagate through the protein. Our results demonstrate, for example, changes in the number of contacts between the switch peptide of TnI and the hydrophobic patch of TnC resulting from mutations in different portions of TnC. We observed that several mutations associated with decreased calcium binding at site II of cTnC resulted in reduced contacts (measured as a proportion of wild type) between the switch peptide and hydrophobic patch (I61Q: 0.96 & D65A: 0.97). Similarly, two mutations associated with DCM also resulted in reduced contacts (I4M: 0.93 & Q50R: 0.91). We also observed that two mutations within the hydrophobic patch and associated with HCM (C84Y: 0.98 & V44M: 0.89) resulted in reduced contacts. Finally, a model mutation in the hydrophobic patch had an increase in contacts (L48Q: 1.04). In addition to these results, we used an enhanced sampling method to estimate how mutations change the affinity of cardiac troponin's regulatory binding site for calcium. Ultimately, affinity changes will inform a spatially explicit model of the half sarcomere, revealing how twitch forces change with alterations to troponin's function.

Probing mutation-induced changes in post-powerstroke conformations of actomyosin via molecular dynamics simulations.

Matthew Carter Childers, Kerry Y. Kao, Sonette Steczina, Saffie Mohran, and Mike Regnier

Department of Bioengineering, University of Washington, Seattle, WA, USA.

Abstract: Post-powerstroke conformations of the actomyosin complex bear force during the chemomechanical cycle and conformational transitions between such states determine relaxation kinetics in striated muscle. These structural transitions are driven by (1) release of ADP from the myosin nucleotide binding pocket, (2) binding of ATP to the myosin nucleotide binding pocket, and (3) detachment of ATP-bound myosin from actin filaments. Hypertrophic cardiomyopathy (HCM) associated mutation G256 and R403Q in cardiac β-myosin have been linked with changes in relaxation kinetics (e.g. $k_{rel,slow}$, $k_{rel,fast}$ $t_{rel,slow}$) of myofibrils prepared from human induced pluripotent stem cell and porcine derived cardiomyocytes relative to wild type controls. Here, we employ molecular dynamics simulations of the ADP-bound and nucleotide actomyosin complex) to investigate the molecular mechanisms by which G256E, and R403Q modify muscle relaxation kinetics. Conventional MD simulations predict that G256E disrupts myosin transducer structure and the conformation of ADP within the nucleotide binding pocket while the R403Q mutation alters the acto-myosin interface. Brownian Dynamics and enhanced sampling MD simulations of ADP release highlight the importance of E228 on nucleotide handling by myosin and predict that mutations altering nucleotide binding pocket structure impair the energetics and structural pathway of ADP release. These simulations collectively demonstrate that mutations can contribute to similar pathologies by adversely affecting multiple structural events in muscle relaxation.

Racial/ethnic disparities in muscle performance and metabolic alterations in men with advanced prostate cancer

Ellen Grewe[1], Lindsey J. Anderson[1,2], Atreya Dash[3,4], Jose M. Garcia[1,2]

[1]Geriatric Research, Education and Clinical Center, [3]Department of Urology; Veterans Affairs Puget Sound Health Care System, Seattle, Washington

[2]Division of Gerontology and Geriatric Medicine-Department of Medicine, [4]Department of Urology; University of Washington, Seattle, Washington

ABSTRACT

Background: Racial/ethnic minority men with prostate cancer (PCa) may experience worse muscle health prior to androgen deprivation therapy (ADT) than White, Non-Hispanic (WNH) men. We hypothesized that non-WNH men would display a worse phenotype, in association with metabolic perturbations, than WNH men initiating ADT.

Methods: We compared Pre-ADT lean mass, performance [hand grip strength, 6-minute walk test, stair climb power, aerobic capacity], and quality of life between WNH (n=40) and non-WNH (n=19); targeted metabolomics (plasma) was also performed (WNH, n=18; non-WNH, n=7). Independent t-tests compared outcomes between groups and spearman correlations tested associations ($p \leq 0.05$). Metaboanalyst v6.0 performed metabolomic analyses with Benjamini-Hochberg adjustment (FDR<0.1).

Results: There was no difference in age, metastases, tumor characteristics, or lean mass; muscle density tended to be worse (p=0.104) in non-WNH. Performance was significantly worse ($p \leq 0.026$) while aerobic capacity (p=0.09) and patient-reported Physical Function tended to be worse (p=0.08) in non-WNH. Plasma Arginine/Proline metabolism was significantly altered (FDR=0.025) while Glycine, Serine, and Threonine metabolism and Glutathione metabolism were nominally altered ($p \leq 0.05$) in non-WNH. Abundance of metabolites related to these pathways was nominally elevated (Hydroxyproline, Creatine, Aminolevulinate) or reduced (Pyroglutamic Acid) in non-WNH ($p \leq 0.05$); Hydroxyproline remained significant (FDR=0.008). Creatine tended to correlate with better stair climb power (r=0.44, p=0.74) in WNH, but not in non-WNH.

Conclusion: Non-WNH men displayed worse performance and altered amino acid/antioxidant metabolism versus WNH men at ADT initiation; worse muscle power may be due to altered creatine cycling. Future research is needed to confirm this and to determine the relevance for ADT-related sarcopenia.

Title: **Characterization of the Role of Muscular AMPK in Sarcopenic Obesity**

Haiming L. Kerr, Kora Krumm, Nornubari Bagia, Ross Burnside, Artur Rybachok, Siyi Jiang, Amanda Chen, Elizabeth Dacek, Lucas Caeiro, Jessica Li, Morgan Sydor, Jose M. Garcia

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

Abstract: Sarcopenic obesity (SO) is characterized by muscle weakness, atrophy, and an increase in body fat with age. While there is currently no FDA-approved treatment for this condition, previous studies have demonstrated an association between reduced levels of muscle AMP-activated protein kinase (AMPK) and aging. Here, we aim to test if AMPK in skeletal muscle is essential for maintaining muscle mass and body composition in aged mice.

We used muscle-specific AMPKα2 transgenic (α2 D157A mutant, TG) mice and compared them to wild-type (WT) mice. Young (4-6 month) and old (20-24) female and male TG and WT mice were evaluated for body composition, grip strength, endurance (treadmill), and muscle mass.

In the young cohort, TG mice of both sexes showed lower treadmill endurance compared to WT. In the old cohort, TG mice of both sexes had decreased muscle mass compared to old WT mice. Across both age groups and genotypes, male mice had a greater difference in body weight and fat mass compared to female mice. Older TG male mice were also more fatigable during muscle physiology tests than WT mice. In contrast, old female mice had a more prominent decrease in physical function and muscle mass with AMPK inactivation compared to male mice.

In conclusion, AMPK is crucial for maintaining endurance in young mice, but in older mice, AMPK is also important for retaining muscle mass and strength while attenuating obesity. Therefore, AMPK may play a critical role in preventing SO with aging.

Investigating the mechanisms of contractile dysfunction of the hypertrophic cardiomyopathy R403Q mutation using a heterozygous and homozygous stem cell derived cardiomyocyte model

Khushi Tawde[1], Steczina, Sonette[2,3], Saffie Mohran[2,3], Tim McMillen[3,4], Kristi Kooiker[3,5], Michael Regnier[2,3]

[1]Department of Biology, University of Washington, Seattle, WA, [2]Department of Bioengineering, University of Washington, Seattle, WA, [3]Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA, [4]Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA, [5]Devision of Cardiology, Department of Medicine, University of Washington, Seattle, WA, USA

Hypertrophic Cardiomyopathy is the most common form of hereditary heart disease affecting ~1:500 individuals, characterized by progressive thickening of the left ventricular wall. The first mutation linked to this disease was the heterozygous R403Q mutation in human beta-myosin heavy chain (β-MHC). Conflicting reports of contractile kinetics between human myectomy samples vs transgenic mouse and rabbit models motivated us to study the molecular mechanisms of altered contraction in a CRISPR/Cas9 gene edited human inducible pluripotent stem cell line. Following differentiation to cardiomyocytes (hiPSC-CMs) and maturation in culture, we isolated sub-cellular contractile organelles called myofibrils. Myofibril contractile kinetics from this line had slowed force development and cross-bridge detachment, with reduced maximal force compared to the WT line. hiPSC-CMs were cast into fibrin matrices to form three-dimensional, engineered heart tissue (EHT) for measures of twitch force and contractile kinetics. At 1Hz stimulation, heterozygous mutation EHT's exhibited a hypercontractile phenotype compared to WT EHTs, with slowed relaxation kinetics. Since the penetrance of our heterozygous R403Q hiPSC-CMs is unknown, we are now studying a homozygous iPSC-CM line where 100% of the β-MHC is mutated. This will allow us to assess the direct contribution of the mutation to the disease contractile phenotype. We will repeat the myofibril and EHT measures of contractile properties and perform stopped flow kinetics analysis on isolated myosin to determine ATP turnover and ATP hydrolysis product release rates. This will provide molecular mechanistic insight of the contractile abnormalities, allowing development of therapeutic interventions that specifically target the mechanisms that alter contractile function.

Novel Therapeutic Approaches for Duchenne Muscular Dystrophy Using Engineered Muscle Tissues and Deimmunized Gene Vectors

P. Barrett[1], K. Louie, C. Le Guiner, L. Maves, A. Smith, S. Luttrell, N. Geisse. J.S. Chamberlain, G.L., Odom and D. Mack.

Although Duchenne Muscular Dystrophy (DMD) is a progressive and degenerative disease there is evidence for embryonic and fetal stage defects during myogenesis, including transcriptional and epigenetic perturbations, suggesting early intervention potential. Two promising treatment approaches have emerged: epigenetic small molecules, which target transcriptional dysregulation, and gene therapy for dystrophin replacement. While epigenetic compounds like Duvyzat show promise in animal models, many have failed in clinical trials. Separately, gene therapies such as Elevidys face distinct challenges, particularly regarding efficacy and safety due to dystrophin-specific T-cell immunity.

Our dystrophin-null EMTs demonstrated characteristic DMD pathology, including force deficits, dysregulated contractile kinetics, and calcium homeostasis disruption. Using these models, we validated the histone deacetylase inhibitor Trichostatin A's efficacy and screened our novel compound EPI1, which showed functional restoration in both EMT and zebrafish models. Additionally, we developed deimmunized micro-dystrophin vectors using in silico prediction tools to minimize immune responses while maintaining functionality. These vectors achieved efficient lentiviral transduction in hiPSC-derived myoblasts with consistent expression across regulatory cassettes.

By combining EMT modeling, high-throughput screening, and computational protein design, we've established a pipeline for optimizing DMD therapeutics. This integrated approach of epigenetic modulation and deimmunized vectors addresses both functional recovery and immune tolerance, potentially expanding treatment accessibility to previously excluded patient populations. Our most promising candidates are advancing to animal studies, representing a significant step toward more effective and safer DMD therapies.

Impact of pathologic cardiac hypertrophy on the regulation of the mitochondrial RNase P splicing complex

Taylor Billings, Rong Tian, and Matthew A. Walker

Decades of research has shown significant energetic crisis in failing hearts. It has been proposed that impaired mitochondrial biogenesis greatly contributes to the energy deficiency and thought to be a pivotal player in the disease progression. Strategies aimed at improving mitochondrial biogenesis in failing heart are urgently needed. Previously, we uncovered a significant defect in the processing of mitochondrial RNA in failing heart that is sensitive to the NAD+/NADH redox state. We speculate that downregulation of one of the critical subunits of mitochondrial RNase P, mitochondrial ribonuclease P protein 2 (Mrpp2), is the culprit. Mrpp2 serves a scaffolding function in RNase P allowing subunit 1 (Mrpp1) to methylate mitochondrial precursor transfer RNA and subunit 3 (Mrpp3) to cleave the 5' end of precursor transfer RNA releasing the adjacent mature messenger RNAs (mRNA). The mature mRNA encodes the thirteen electron transport chain proteins responsible for mitochondrial oxidative phosphorylation. In the present study, we found that isoproterenol induced cardiomyocyte hypertrophy downregulated Mrpp2 and suppressed mitochondrial N^1 adenosine methylation in differentiated H9C2 cells. N^1-methyladenosine is a prominent post-transcriptional modification introduced to mitochondrial RNA by Mrpp1 when complexed with Mrpp2 in RNase P. These modifications stabilize mitochondrial transfer RNA and are crucial for translation of the mitochondrial proteome. The results suggest that downregulation of Mrpp2 in pathologic cardiac hypertrophy disrupts the mitochondrial RNase P complex thereby dysregulating methylation of mitochondrial RNAs. The next step will be to replenish Mrpp2 levels in pathologic cardiac hypertrophy and determine if mitochondrial RNA methylation is restored.

Title: Assessing myocyte–targeted reversibility of inherited dilated cardiomyopathy

Bella Reichardt

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, the limits of cardiac reverse remodeling have not been well defined in DCM. To assess the potential of a myocyte-targeted therapy 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 of the myofilament 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 remodeling, including excess ECM deposition and increased fibroblast number, was not reversible and may have actually worsened with DOX. To determine if there is ever 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.

Measuring myofilament specific calcium in HIPSC cardiomyocytes with improved optogenetic sensors.

(Graduate Student) Anthony Asencio[1], Justin D. Lee[2], Sarah Wait[2], Michael Regnier[3], Andre Berndt[1], Farid Moussavi-Harami[4].

[1]BioEngineering, University of Washington, Seattle, WA, USA, [2]Molecular Engineering, University of Washington, Seattle, WA, USA, [3]Dept BioEngg, Univ Washington, Seattle, WA, USA, [4]Cardiology, Univ Washington, Seattle, WA, USA.

Genetic cardiomyopathy can emerge from point mutations in sarcomeric proteins. To understand disease progression and develop targeted treatments, a better understanding of the affected molecular mechanisms regulating contraction is needed. New experimental tools have been developed that can assist in this regard. First, human cardiac cells and tissue derived from induced pluripotent stem cells (iPSCs) have become valuable models for studying the initiating events in genetic cardiomyopathies. Second, novel calcium indicators and localization strategies allow for time resolved compartmental measurements of ionic calcium. These strategies target cardiac troponin I or T (cTnT or cTnI). We aimed to improve upon the strategy of using fusion proteins to better quantitate measurements of the free calcium concentration in myofilaments using the green protein calcium sensor GCAMP6F fused to the n-terminus of cTnT. To estimate the local effect of decreased myofilament calcium buffering, we will lipofect our DNA construct into cells differentiated from three hiPSC lines that have different troponin C (TnC) calcium binding affinities. These cell lines have the WTC11 iPSC background and express: WT TnC, heterozygous cTnC I61Q (with reduced calcium binding), and homozygous cTnC D65A (that does not bind calcium in the N-terminal site II). Our preliminary results show that our probe tagged cTnT localizes the areas of the sarcomere filament with troponin as expected, the sensor brightness is improved over other fused protein probes, and the on/off rates are satisfactory for estimating myofilament calcium kinetics.

A canine model of cholestasis after AAV gene therapy for myotubular myopathy

Amy Duong, SiWei Luo, Violet Phan, Emanuela Pannia, Nika Maani, James Dowling, Julie Crudele, David Mack

Abstract

X-linked myotubular myopathy (MTM) is a form of centronuclear myopathy resulting in muscle weakness and hypotonia, with critical infant cases experiencing respiratory distress and feeding difficulties. ASPIRO is a recent MTM adeno-associated viral vector (AAV) gene therapy trial where four patients died due to liver failure secondary to cholestasis, likely triggered by the administered high dosage of AAV. For our study, we used a dog model to investigate the correlation between MTM and hepatobiliary disease to model the adverse events of underlying hepatic elements seen in humans. A 12 week old wildtype and an MTM male puppy were fed a specialized diet to induce cholestasis. At 14.5 weeks old, they were infused with 97% empty capsid AAVs; the total AAV administered was the capsid equivalent of 7.33E+14 vg/kg. H&E stains show bile accumulation in the MTM puppy liver while elevated expression of the bile salt export pump (BSEP) was seen via western blots. Future assays include a western blot for MDR3 (multidrug resistance protein 3, which secretes phospholipids from hepatocytes into bile) and blinded scoring to evaluate if the bile accumulation is related to the MTM genotype, special diet, and/or administration of AAV. The study aims to provide groundwork for utilizing large animal models to test cholestasis treatments, enteral feed formulas, and/or modified AAVs to effectively reduce harm with AAV gene therapies in MTM patients.

A Computational and Experimental Investigation of the Dysfunction of the Dilated Cardiomyopathy-Associated MYH7 Mutation R369Q

Aditi M. Prabhala, Matthew C. Childers, Kerry Y. Kao, Timothy S. McMillen, Michael Regnier

*Presenting author is a graduate student

Mutations in the sarcomeric protein β-myosin heavy chain (MHC) are commonly implicated in familial cardiomyopathies. Such mutations can lead to progressive remodeling of cardiac muscle, significantly impairing the heart's function. Our lab aims to understand the mechanisms by which these mutations contribute to disease. One mutation of interest, R369Q, resides on loop 4 of the β-MHC motor domain and is associated with dilated cardiomyopathy. In molecular dynamics simulations of pre-powerstroke myosin, we showed that the overall structure of R369Q β-MHC is less structurally stable and has altered contacts that could impact structural signal transduction between functional regions of myosin. Additionally, recent work by our group and collaborators using targeted molecular dynamics has suggested that the R369 residue is involved in repelling tropomyosin during the C-state to M-state transition, promoting strong actomyosin binding. Experimentally, we leveraged a CRISPR-Cas9 gene-edited homozygous *MYH7*$^{R369Q/R369Q}$ human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) model. Preliminary measurements of contraction and relaxation kinetics from myofibrils revealed a higher specific force, decreased duration of the slow phase of relaxation, and increased rate of the fast phase of relaxation in R369Q myofibrils. More rapid relaxation kinetics imply faster crossbridge cycling, suggesting that ADP or phosphate release could be accelerated. To further examine how the R369Q mutation contributes to pathological contractile dysfunction, future work will involve modeling the post-powerstroke state using molecular dynamics and cryo-EM to interrogate the mutation's effect on nucleotide release and performing additional myofibril measurements at submaximal calcium to gain further insight into changes in thin filament activation.