Session 4. Small molecule modulators of sarcomere protein function

Lectures

L4.1

Modulating the cardiac sarcomere to develop precision medicine for different classes of heart diseases – the story of mavacamten and danicamtiv

Suman Nag

Biochemistry and Biophysics, Cardiovascular-West, Bristol Myers Squibb
Suman Nag <suman.nag@bms.com>

Many heart diseases such as hypertrophic cardiomyopathy (HCM) and dilated. Many heart diseases such as hypertrophic cardiomyopathy (HCM) and dilatedcardiomyopathy (DCM) are engendered by aberrant cardiac muscle contractility, which is often, if not always, caused by abnormal sarcomere function. Many cases of HCM and DCM are familial, arising from genetic mutations in certain essential sarcomeric proteins such as myosin, myosinbinding protein-C, and titin. Studying the pathophysiology of such heart diseases – fromfundamental biochemical principles to animal models and to the clinic – allows us to understandthe underlying causal biology of the disease itself and to pursue precision-based therapeutics. Mavacamten and danicamtiv, both clinical-stage molecules that MyoKardia Inc. (recently acquiredby Bristol Myers Squibb) designed to treat targeted segments of HCM and diseases of impairedsarcomere contractility, respectively, are living examples of how modulating the cardiac sarcomerecould potentially lead to targeted therapies for cardiovascular diseases. In this seminar, we willsurvey the primary mechanisms of action of these small molecules on cardiac sarcomeric myosinand explain the molecular underpinnings of their potential therapeutic benefits.

L4.2

Muscle diseases: from molecular mechanisms to new treatments

Louise Canon, Julien Robert-Paganin, Carlos Kikuti, Daniel Auguin, Anne Houdusse

Structural Motility, Institut Curie, Paris Université Sciences et Lettres, Sorbonne Université, CNRS UMR144, Paris, France
Anne Houdusse <anne.houdusse@curie.fr>

Hypertrophic and dilated cardiomyopathies (HCM and DCM) are highly prevalent cardiac diseases. They result from distinct single-point mutations in sarcomeric proteins that lead to muscle dysfunction. Most of the current treatments for end-stage cardiomyopathies such as heart transplantation or implantable-cardioverter are highly invasive. Recently, a new approach using small-molecules able to modulate myosin force production has been proposed to treat cardiac disease. Some of these small molecules such as the activator Omecamtiv mecarbil (OM) and the inhibitor Mavacamten (Mava) are currently in advanced clinical trials. Aficamtem is another specific cardiac myosin inhibitor currently in phase 2 clinical trials, while MPH-220 is specific for skeletal muscle myosin. In this study, we used a combination of X-ray crystallography and molecular dynamics in order to decipher the mechanism of action of these drug candidates. Our results describe the binding pocket of these drugs and highlight the basis of their specificity. The comparative study of the mode of action of an activator and an inhibitor of Pi release in cardiac myosin provides the blueprint for allosteric modulation of force production by a myosin, but also opens the road to the design of new treatments.

L4.3

High throughput screening for cardiac troponin activators and inhibitors

Thomas Kampourakis

Randall Centre for Cell and Molecular Biophysics, and British Heart Foundation Centre of Research Excellence, King's College London, London, UK
Thomas Kampourakis <thomas.kampourakis@kcl.ac.uk>

Current therapeutic interventions for both heart disease and heart failure are largely insufficient and associated with undesired side effects. Biomedical research has emphasized the role of sarcomeric protein function for the normal performance and energy efficiency of the heart, suggesting that directly targeting the contractile myofilaments themselves using small molecule effectors has therapeutic potential and will likely result in greater drug efficacy and selectivity. We exploited recent advances in understanding the regulatory structural transitions in the myofilaments of heart muscle and developed robust high-throughput screening platforms that will allow us to identify potential lead compounds to treat heart failure. We used a range of newly developed fluorescence polarization assays, composed of fluorophore-labelled peptides of cardiac troponin I that specifically bind to cardiac troponin C in a calcium-dependent manner, to identify small molecule effectors of the thin filament activation pathway. We screened a series of commercially available small molecule libraries and identified several hit compounds with both inhibitory and activating effects. Hits identified in the primary screens were validated, and their mechanism of action characterized using a wide range of biochemical and biophysical techniques. The current studies underpin the rational design of compounds with either calcium sensitizing or desensitizing properties that create the mechanistic basis for the development of new small molecule effectors to treat heart failure.

Acknowledgements of Financial Support: Supported by the British Heart Foundation.

Oral Presentations

O4.1

Piperine-derived compounds modulate in both directions skinned fibers resting ATPase activity

P. A. Bermudez Mora[1,2], A. G. Dumitras[1,2], A. Emanuelli[1,2], L. Cavallini[3], B. Blaauw[1,2], M. Sturlese[3], A. Mattarei[3], L. Nogara[1,2]

[1]Department of Biomedical Sciences, University of Padua, Padua, Italy; [2]Veneto Institute of Molecular Medicine (VIMM), Padua, Italy; [3]Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy
Leonardo Nogara <leonardo.nogara@unipd.it>

The resting state of myosin (SRX) has important implications on both regulations of muscle contraction and the energetic balance of muscle tissue. Due to a large amount of skeletal muscle tissue in the body, the stability of the SRX has a potentially large impact on the overall body basal metabolism. We recently demonstrated that piperine, an alkaloid contained in black pepper seeds, destabilizes the SRX structure, increasing resting ATPase activity of skinned skeletal muscle fibers. To improve piperine efficacy, we developed a library of about 30 new piperine-derived compounds and screened them for their ability to affect the ATPase activity of rabbit psoas fibers. In this work, we developed a new 384-wells plate single fibers ATPase assay to measure the concentration/activity relationship, we evaluated the effect of these molecules on fiber tension, and we identified which components of the molecules are responsible for binding by NMR analysis.
Few molecules induced a higher ATP consumption compared to piperine, and, interestingly, some molecules were able to decrease ATP hydrolysis. Two SRX destabilizers and two SRX stabilizers have been characterized in HMM showing no effect on purified myosin and on slow soleus fibers, confirming the piperine-inherited specificity for fast myosin isoforms. Importantly, these molecules showed a smaller but consistent effect on human fibers.

O4.2

Diastolic dysfunction in a rat COPD model: Impact of β-adrenergic blockade on cardiac features

P. E. Grillet[1,2], C. Reboul[3], P. Bideaux[1], A. Bourdin[1,4], O. Cazorla[1]

[1]PhyMedExp, INSERM U1046 - CNRS UMR 9214 - Montpellier University, Montpellier, France; [2]Biochemistry and Hormonology Department, Montpellier University Hospital, Montpellier, France; [3]LaPEC EA4278, Avignon University, Avignon, France; [4]Department of Respiratory Diseases, Montpellier University Hospital, Montpellier, France
Pierre-Edouard GRILLET <pe.grillet@gmail.com>

Introduction: Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of death worldwide caused by smoke exposure. This leads to an airflow limitation and emphysema and is worsened by exacerbations mainly due to pulmonary infections. Cardiac dysfunction is a common feature of the disease suggesting a crosstalk between pulmonary and cardiac function.We aim to understand the early modifications leading to cardiac dysfunction in an animal model that mimics COPD.
Methods: We developed a rat model of elastase-induced emphysema with pulmonary septic exacerbation (ELA-LPS). Pulmonary and cardiovascular functions were explored *in-vivo* and *ex-vivo*. Excitation-contraction coupling and sarcomere mechanics were studied in isolated cardiomyocytes.
Results: ELA+LPS lead to emphysema and airflow obstruction. Animals exhibit cardiac hypertrophy and diastolic dysfunction with preserved ejection fraction assessed by echocardiography. ECG reveals tachycardia and rhythmic variability abolition along with sympathic stimulation.
At cellular level, LV isolated cardiomyocytes present hypercontractility as calcium transients increased. Furthermore, higher passive tension in sarcomeres without modification of calcium sensitivity is observed, suggesting relaxation alteration. Utilization of ß-blocker bisoprolol reverses most of alterations: rhythm, diastolic dysfunction, calcium coupling.
Conclusion: ELA-LPS animal model expresses feature of COPD associated to HFpEF. Pathogenesis seems to be associated with β-adrenergic stimulation, confirmed by ß-blocker treatment recovery. Analysis of molecular effectors could highlight specific targets to treat COPD patients.

O4.3

MPH-220, a first-in-class anti-spastic drug candidate efficiently relaxes spastic muscles by direct skeletal muscle inhibition

M. Gyimesi[1,2,3] A. I. Horvath[1], D. Turos[1], Mate Penzes[1], Louise Canon[4], Carlos Kikuti[4], A. A. Rauscher[2], M. Kovacs[1], Anne Houdusse[4], A. Malnasi-Csizmadia[1,2,3]

[1]MTA-ELTE Motor Pharmacology Research Group, Pazmany Peter setany 1/c, 1117 Budapest, Hungary; [2]Motorpharma, Ltd., Szilagyi Erzsebet fasor 27, 1026 Budapest, Hungary; [3]Printnet, Ltd., Kisgomb utca 25-27, 1135 Budapest, Hungary; [4]Structural Motility, Institut Curie, Paris Universite Sciences et Lettres, Sorbonne Universite, CNRS UMR144, 75005 Paris, France
Andras Malnasi-Csizmadia <malna@elte.hu>

Muscle spasticity disables self-supporting life management of 60 million patients with various nervous system injuries including stroke, MS, TB, SCI and cerebral palsy. There is a high unmet medical need for an efficient antispastic drug because current muscle relaxants are often of limited efficacy and cause severe neurological and cardiovascular side effects due to targeting the central or peripheral nervous system. We developed a new-generation anti-spastic oral drug candidate, MPH-220, which efficiently relaxes spastic skeletal muscles and lacks cardiovascular and neurological adverse effects due to its mechanism of action: MPH-220 selectively inhibits fast skeletal myosin 2 isoforms without having any effect on cardiac myosin, as evidenced by myosin samples from human biopsies. Moreover, through direct muscle relaxation, brain injury induced spastic gait disorders were significantly improved in an animal disease model after oral administration of MPH-220 without cardiovascular and neurological side effects. Due to its mechanism of action supported by the co-crystal structure of MPH-220 and skeletal muscle myosin 2, MPH-220 does not cause complete loss of muscle tone even at high doses, which is a great advantage over the currently used muscle relaxants. Furthermore, MPH-220 has excellent ADMET properties for human oral administration demonstrated by the comprehensive pre-clinical toxicology studies performed on three species: it is highly absorptive, selectively accumulates in skeletal muscle tissues enabling low-dose daily treatment and causes no adverse effects in large animals even at extremely high doses.

O4.4

Omecamtiv mecarbil modulation of force generation in human cardiac muscle

B. Scellini[1], N. Piroddi[1], M. Dente[1], G. Vitale[1], R. Coppini[2], C. Ferrantini[1], C. Poggesi[1], C. Tesi[1]

[1]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [2]Department of Neurofarba, University of Florence, Florence, Italy

Beatrice Scellini <beatrice.scellini@unifi.it>

Omecamtiv mecarbil (OM) is a small molecule proved to enhance the function of the beta-myosin motor and presently used for the treatment of heart failure. Despite positive outcomes of clinical trials, the mechanistic basis of its action is controversial and little is known of its functional impact in human cardiac sarcomeres. Here myofibrils from human donor left ventricle have been used to study the effects of μmolar [OM] on isometric force in relaxing conditions (pCa 9) and at maximal (pCa 4.5) or half-maximal (pCa 5.75) calcium activation, both in control conditions (15°C, equimolar DMSO, contaminant [Pi] ~ 170 μM) or in presence of 5 mM [Pi]. In all conditions tested, OM increased isometric force with a complex dose-dependent effect peaking (about 40% increase) at 0.5 μM and strongly depressed the kinetics of force development up to 90% at 10 μM OM. OM was also found to decrease the inhibition of force operated by Pi at low (1-2 mM) [Pi], in agreement with observations in rabbit slow-muscle (Governali *et al.*, 2020). OM induced calcium-independent force development which approached (with a very slow dose-independent kinetics) the level of calcium activated force at 10 μM OM. The results obtained in human cardiac myofibrils and multicellular preparations are in agreement with OM operating a complex perturbation of the thin/thick regulatory state of the sarcomere mediated by binding to allosteric sites coupled to Pi release.

Acknowledgements of Financial Support: SilicoFCM EU H2020 grant agreement n. 777204.

Virtual Posters

P4.1

Structure of the asynchronous flight muscle thick filament from the bumble bee, *Bombus ignitius*, at 6 Å Resolution

Jiawei Li[1], Hamidreza Rahmani[1], Fatemeh Abbasi Yeganeh[1], Hosna Rastegarpouyani[1], Dianne W. Taylor[1], Hiroyuki Iwamoto[2], Kenneth A. Taylor[1*]

[1]Florida State University, Institute of Molecular Biophysics, Tallahassee, FL 32306-4380, USA; [2]JASRI, Spring8, Japan

Jiawei Li <jl18n@my.fsu.edu>

Asynchronous flight muscle empowers insects to achieve high wingbeats and save energy. The bumble bee, *Bombus ignitus* (order Hymenoptera), has developed this mechanism. To what extent are its thick filaments similar to those of *Drosophila* (order Diptera) and *Lethocerus* (order Hemiptera)? To answer this question, we obtained a cryo-EM structure at 6 Å resolution of isolated *Bombus ignitus* flight muscle thick filaments. Myosin tails of *Bombus* are very similar to those of *Lethocerus* and *Drosophila*, with a very similar packing into "ribbons" and a similar helical angle. Myosin heads of *Bombus* thick filaments are disordered in relaxed muscle, similar to *Drosophila*, but not for the same reason as there are no non-myosin proteins on the *Bombus* filament surface, unlike *Drosophila.* The tail structure at three of the four skip residues is very similar, but Skip 1 adopts a visible difference in orientation with respect to the backbone. Non-myosin densities from flightin and myofilin in *Bombus* are greater than either *Lethocerus* or *Drosophila*, and more paramyosin is visible than in *Drosophila*. The greater density attributable to flightin and myofilin may explain both the myosin head disorder as well as the unusual high salt insensitivity of *Bombus* thick filaments.

Acknowledgements of Financial Support: Supported by NIH/NIGMS.