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CYTOSKELETON NEWS

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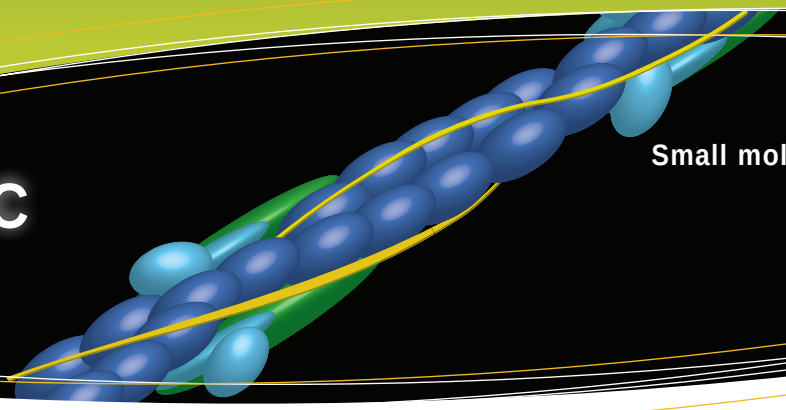
NOV/DEC 2012



this issue

Upcoming Meetings

ASCB 2012
San Francisco, CA, USA
Booth # 901
Dec. 15-19, 2012

SLAS 2013
Orlando, FL, USA
Booth # 2031
Jan. 12-16, 2013

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Small molecule modulators of myosin

Background

The chemical intervention of myosin has lagged behind other classes of drug targets because of a reluctance to focus on an intracellular molecule which is essential for all human cell types and because in muscle cells it has a high concentration which could potentially reduce the effects of a drug. In the last ten years this situation has changed with the realization that a compound can infiltrate deep into dense muscle tissue and that the myosin family's diversity allows for quite targeted modulation.

The myosin superfamily is constantly being revised, with one of the latest versions describing 37 classes based on structural domain phylogenies[1]. Of these 37 classes, twelve are present in humans and in these twelve classes there are 39 human genes. Of particular significance are the molecular motors involved in cardiac, skeletal and smooth muscle functions which are myosins of Class II; however, there are many more targets that are also of medical value such as myosins of the inner ear (VII), skin (Va) and intestinal microvilli (Ia) (Table I).

Table 1 – Small molecule modulators of myosin

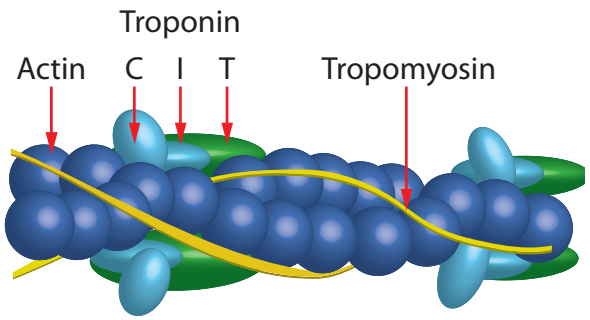
Myosin	Modulator	Mechanism	Disease relevance	Ref #
All Class II isoforms	Blebbistatin	Binds to myosin and inhibits the force cycle at the weakly bound state	not determined	2,3,4
Cardiac II	CK1122534 and Omecamtiv mecarbil	Binds at the cleft between the force generating do-main and the ATP binding pocket. Increases the time in the strongly bound state	Heart disease; systolic dysfunction	5,8
Non-muscle II	Ammosamides A&B	Binds irreversibly to an unknown site	not determined	11
Smooth II	CTK2018448	Inhibits myosin and improves renal blood flow	Hypertension	10
Non-muscle Va	Manassantin B	Inhibits the interaction between Myosin Va and melanophillin	Skin coloration disorders	12

Isoform specific myosin drugs

Blebbistatin was one of the earliest identified specific myosin inhibitors and was found to inhibit some class II myosin isoforms with different IC50s[2-4]. In particular, it has 0.5 to 80 µM affinity for myosin II isoforms[4]. This report opened up the possibility that compounds could target different isoforms of myosin, and soon after reports emerged that identified several compounds with high affinity for the cardiac myosin II isoform[5] and smooth muscle myosin II isoform[6]. One of these, omecamtiv mecarbil (OM), has been the focus of drug development programs which have reached Phase II clinical trials (clinicaltrials.gov).

It's important to consider other targets within the sarcomere that interact with myosin and may affect its response to new drugs. In particular, the tropomyosin and troponin C, T, and I complex (T4 complex, Fig. 1) are mediators of the calcium signal for muscle contraction. In concert with the other three members of this complex, Troponin C binds calcium which reduces its affinity for F-actin which allows myosin to bind F-actin and generate force. In an assay format, this has the dramatic effect of increasing the release of phosphate after ATP hydrolysis by up to 100 fold. A selection of these reagents is available from Cytoskeleton on a custom basis (Table 2).

Figure 1: Muscle thin filament structure



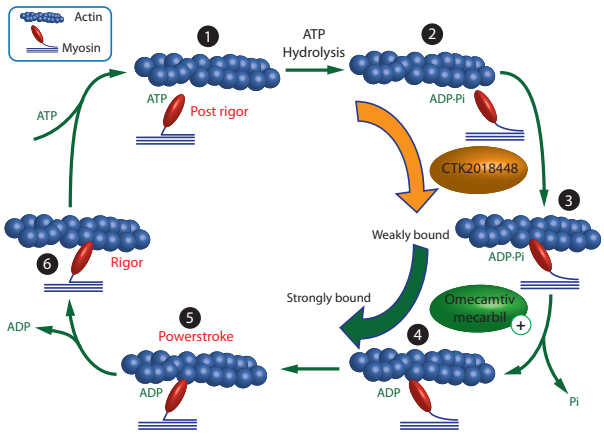
Pharmacology

Pharmacologically, myosin is an interesting molecule to study because it has many structural states that constitute the myosin force or duty cycle[7] (Fig. 2). In particular, OM has been shown to affect the length of time in the strong force part of the cycle (Fig. 2, green arrow), which is thought to underlie its ability to prolong

Continued from Page 1

systolic contraction. Interestingly, diastolic contraction is not affected by OM[8]. This is rather unexpected but clinically useful because heart failure has a predominance of reduced systolic contraction[9]. In a similar vein, the smooth muscle inhibitor CTK2018448 (acts in the weakly bound part of the duty cycle; Fig. 2, orange arrow) is reported to increase renal blood flow but not limb blood flow, which is useful for hypertensive patients who have renal insufficiency[10]. These findings indicate that myosin modulators can have useful and somewhat unpredicted manifestations in whole body systems compared to *in vitro* assays.

Figure 2: Actin and Myosin Interactions



Custom Modules

Table 2 – Custom modules from Cytoskeleton

Item	Use	Aliquot size (mg)	Cat #
Cardiac Myosin II S1 fragment	High activity subunit of cardiac muscle myosin for ATPase assays	inquire	na
Smooth Myosin II S1 fragment	High activity subunit of smooth muscle myosin for ATPase assays	inquire	na
Skeletal Myosin II S1 fragment	High activity subunit of skeletal muscle myosin for ATPase assays	inquire	na
Cardiac Tropomyosin/ Troponin complex	Contains tropomyosin, troponin T, troponin C and troponin I in equimolar amounts. Creates calcium sensitive myosin ATPase assay	inquire	na
Myosin ATPase assays as a service	To provide accurate and efficient screening data	Inquire	na
Actin (cardiac)	F-actin subunit for myosin ATPase assays	1 mg	AD99
Actin (skeletal)	F-actin subunit for myosin ATPase assays	1 mg	AKL95 or AKL99
Myosin (rabbit skeletal)	Myosin for skeleton muscle ATPase assays	1 mg	MY02
Myosin (bovine cardiac)	Myosin for cardiac muscle ATPase assays	1 mg	MY03

Note – For more information please contact tservice@cytoskeleton.com

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