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CYTOSKELETON NEWS

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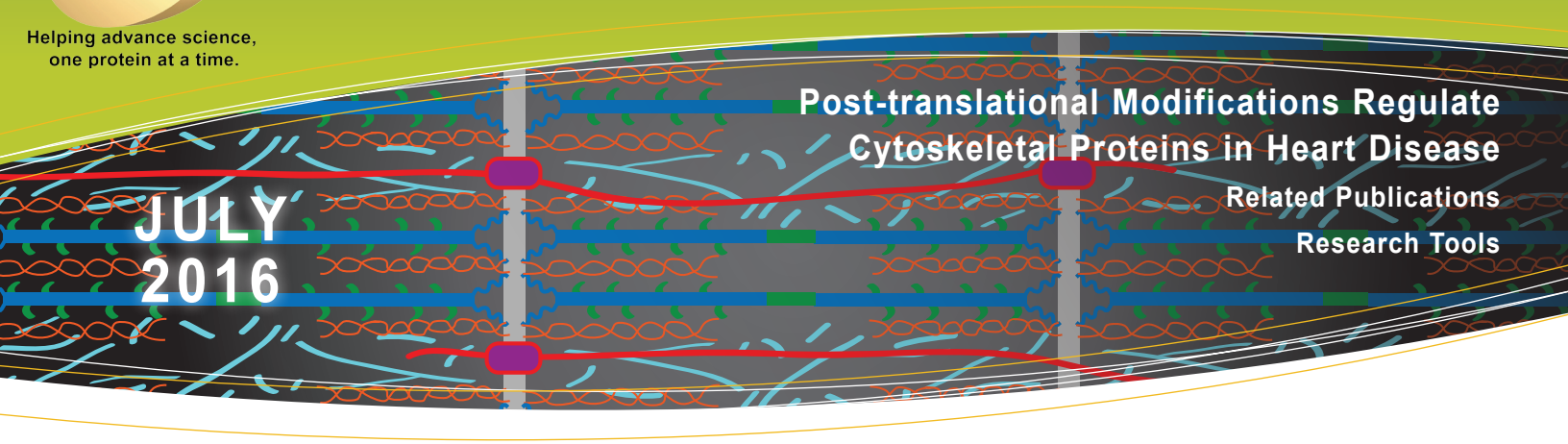
JULY 2016



Post-translational Modifications Regulate Cytoskeletal Proteins in Heart Disease

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PTMs Regulate Cytoskeletal Proteins in Heart Disease

Cardiovascular disease accounts for roughly one in every three deaths in the USA with heart disease accounting for the majority of these cases[1]. The pathology of heart disease often involves the death or dysfunction of cardiomyocytes, specialized heart cells that produce the contractile, beating function of the heart. Many different proteins and cell machinery, such as ion channels and pumps, cytoskeletal proteins, and receptors play a significant role in regulating the contractile ability of cardiomyocytes. Interestingly, many of these proteins are regulated through post-translational modifications (PTMs), in part because PTMs allow for rapid, but subtle changes to a protein as part of an overall cellular response[2]. For example, SUMOylation of the critical sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a) pump was diminished in failing human heart samples[3]. Interestingly, restoration of SUMOylated SERCA2a was sufficient to repair cardiomyocyte function, implicating SUMOylated SERCA2a as a potential target for therapeutic intervention. Indeed, N106, a recently developed small molecule that triggers and maintains SUMOylation of SERCA2a via activation of the SUMO-activating enzyme E1 ligase, improved ventricular function in mice with heart failure[4]. There are many other examples of PTM-modified proteins playing a critical role in cardiomyocyte function and progression of heart failure[2]. Here, we will highlight three examples where post-translationally modified cytoskeletal proteins facilitate cardiomyocyte contraction and normal heart function.

Microtubules: α-tubulin

Microtubules (MTs) play several roles in cardiomyocytes including functioning as compression-resistant elements, performing transport functions, and relaying signaling by converting contractile forces to intracellular signals[5]. MTs are formed by the polymerization of α/ß-tubulin heterodimers. In particular, the c-terminal tail of α -tubulin is heavily modified by PTMs including glutamylation, acetylation, and detyrosination[6]. Several studies have linked tubulin PTMs to progression of human disease. There is a particular focus on detyrosination of α-tubulin, the cleavage of the C-terminal tyrosine residue on the α-tubulin tail. Detyrosination increased cytoskeletal stiffness and altered cardiomyocte function as this modification affects MT-based mechanotransduction[7]. Use of SiR-tubulin technology to perform high-speed, sub-diffraction imaging revealed that detyrosination of α-tubulin was critical for anchoring MTs to sarcomeres in order to regulate MT buckling during contraction[8] (Fig. 1). Furthermore, detyrosination was significantly increased in patients with clinically diagnosed hypertrophic and dilated cardiomyopathies, while acetylation and glycosylation of α-tubulin did not play a significant role in MT buckling[8]. It will be interesting to determine which PTMs regulate alternative functions of MTs in heart disease.

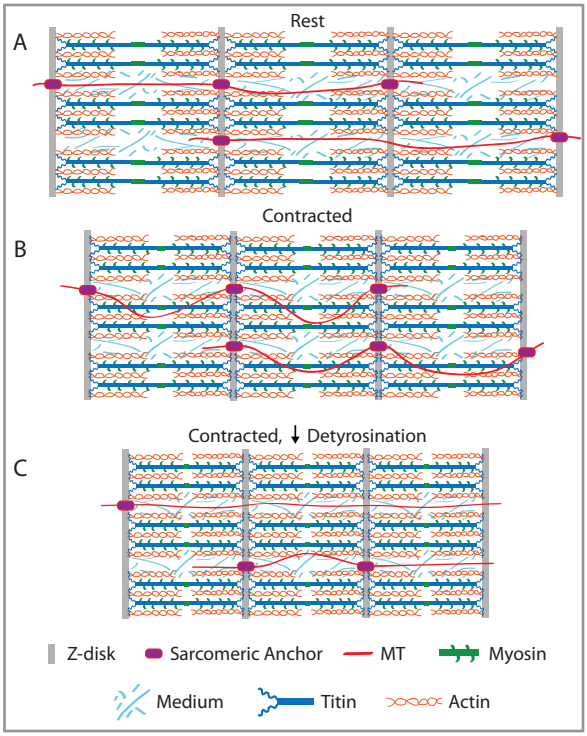

Fig 1. Diagram of MTs attached to sarcomeres in a cardiomyocyte under resting conditions (A). In response to systolic contraction, MTs buckle to accommodate the changing geometry of the myocyte (B). When MT detyrosination is reduced, attachment to the sarcomere decreases, resulting in a sliding rather than buckling action of the MT (C).

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Intermediate Filament: Desmin

Desmin is a muscle-specific intermediate filament protein that forms a scaffold for the contractile machinery in cardiomyocytes, and desmin mutations play a significant role in various heart diseases and are classified as desmin-related myopathies (DRM)[5]. Desmin is a highly modified protein, undergoing phosphorylation, ubiquitination, and ADP-ribosylation, among other PTMs[9]. Importantly, these modifications are implicated in desmin dysfunction and progression of muscle-related diseases. For example, in myofibrils prepared from dissected muscles of food-deprived mice, desmin is phosphorylated, which marks it for subsequent ubiquitination and degradation, decreasing desmin protein levels[10]. It is unknown whether this mechanism occurs in cardiomyocytes under pathologic conditions; however, other studies identified phosphorylation of desmin as a modification altered in heart disease[11]. Because desmin is so highly modified by PTMs, it will be interesting to determine how important PTM crosstalk is for regulating desmin expression, cleavage, and function as it relates to heart disease.

Troponin complex: Cardiac Troponin I (cTnI)

Activity of the trimeric troponin complex is controlled by calcium and in turn the complex regulates tropomyosin's position on actin thin filaments and is a key regulator of sarcomere contraction. One component of the troponin complex, cardiac troponin I (cTnI), functions as a critical regulator of sarcomere contraction, and is an important biomarker of heart disease due to its degradation and appearance in the blood[12]. Phosphorylation of cTnI at several sites is thought to affect troponin complex formation and cardiomyocyte function, and is altered in human heart disease[12]. Little is known about other potential PTMs of cTnI; although, a proteomic study on purified cTnI from human heart showed that cTnI can also be acetylated, oxidized and/or cleaved, in addition to being phosphorylated[13]. However, this study may have missed other modifications such as ubiquitination or SUMOylation as the analysis was performed on a single gel band. Whether these lesser known or yet to be identified PTMs of cTnI regulate its function in heart disease remains unknown; however, these PTMs are deserving of intensive research as cTnI is a critical disease biomarker.

Conclusions

This newsletter discusses the importance and prevalence of PTMs on cytoskeletal proteins and how they can have significant effects on human health and disease like heart failure. Importantly, the emergence of PTMs as potential therapeutic targets for treatment of heart disease is highlighted. With the development of novel tools to identify new PTM targets, it is much more feasible to discover novel PTM dependent mechanisms regulating cytoskeletal proteins. To assist researchers, Cytoskeleton, Inc. offers Signal Seeker™ Kits designed to analyze post-translationally modified proteins under endogenous, physiologic conditions, which are critical to gain a meaningful picture of their role in disease progression.

Custom Proteins

Unlabeled Actins	Source	Purity	Amount	Cat. #
Actin Thin Filaments (calcium sensitive tropomyosin, troponin C, I , T, and Actin Complex)	Bovine cardiac muscle	90%	1 x 1 mg	CS-TFC01
Ebashi Complex (complex of tropomyosin, troponin C, I, and T)	Bovine cardiac muscle	70%	1 x 1 mg	CS-TT05
Myosin - cardiac S1 fragment	Bovine cardiac muscle	90%	1 x 250 µg	CS-MYS03-A

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NEW Signal Seeker™ Kits

Product	Amount	Cat. #
NEW Signal Seeker™ Phosphotyrosine Enrichment Kit	30 Rxs	BK160
NEW Signal Seeker™ Ubiquitin Enrichment Kit	30 Rxs	BK161
Acetyl Lysine Antibody Mouse Monoclonal Validated in WB, IP, IF, ChIP	2 x 100 µl 1 x 25 µl	AAC01 AAC01-S
Phosphotyrosine Antibody Mouse Monoclonal Validated in WB, IP, IF	2 x 100 µl 1 x 25 µl	APY03 APY03-S
Phosphotyrosine Affinity Beads Validated in WB, IP, IF, ELISA	4 x 300 µl	APY03-Beads
Phosphotyrosine-HRP Antibody Mouse Monoclonal Validated in WB	1 x 100 µl 1 x 25 µl	APY03-HRP APY03-HRP-S
SUMO-2/3 Mouse Monoclonal Antibody Validated in WB, IP, IF	2 x 100 µl 1 x 25 µl	ASM23 ASM23-S
SUMO-2/3 Mouse Monoclonal Antibody Validated in IP, IF	2 x 200 µl 1 x 150 µl	ASM24 ASM24-S
SUMO-2/3 Affinity Beads Validated in IP	2 x 400 µl	ASM24-Beads
Ubiquitin Antibody Mouse Monoclonal Validated in WB, IF	2 x 100 µl 1 x 25 µl	AUB01 AUB01-S

Purified Actin

Unlabeled Actins	Source	Purity	Amount	Cat. #
Actin Protein	Rabbit skeletal muscle	>99%	4 x 250 µg 2 x 1 mg 5 x 1 mg 10 x 1 mg 20 x 1 mg	AKL99-A AKL99-B AKL99-C AKL99-D AKL99-E
Actin Protein	Rabbit skeletal muscle	>97%	1 x 1 mg 5 x 1 mg	AKL95-B AKL95-C
Actin Protein	Bovine cardiac muscle	>99%	1 x 1 mg 5 x 1 mg	AD99-A AD99-B
Actin Protein	Smooth muscle, chicken gizzard	>99%	1 x 1 mg 5 x 1 mg	AS99-A AS99-B
Actin Protein	Human platelet, non-muscle	>99%	2 x 250 µg 1 x 1 mg 5 x 1 mg	APHL99-A APHL99-C APHL99-E
Pre-formed Actin Filaments	Rabbit skeletal muscle	>99%	1 x 1 mg 5 x 1 mg	AKF99-A AKF99-B