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Functions of Myosin Light Chain-2 (MYL2) In Cardiac Muscle and Disease

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Abstract

Myosin light chain-2 (MYL2, also called MLC-2) is an ~19 kDa sarcomeric protein that belongs to the EF-hand calcium binding protein superfamily and exists as three major isoforms encoded by three distinct genes in mammalian striated muscle. Each of the three different MLC-2 genes (MLC-2f; fast twitch skeletal isoform, MLC-2v; cardiac ventricular and slow twitch skeletal isoform, MLC-2a; cardiac atrial isoform) has a distinct developmental expression pattern in mammals. Genetic loss-of-function studies in mice demonstrated an essential role for cardiac isoforms of MLC-2, MLC-2v and MLC-2a, in cardiac contractile function during early embryogenesis. In the adult heart, MLC-2v function is regulated by phosphorylation, which displays a specific expression pattern (high in epicardium and low in endocardium) across the heart. These data along with new data from computational models, genetic mouse models, and human studies have revealed a direct role for MLC-2v phosphorylation in cross-bridge cycling kinetics, calcium-dependent cardiac muscle contraction, cardiac torsion, cardiac function and various cardiac diseases. This review focuses on the regulatory functions of MLC-2 in the embryonic and adult heart, with an emphasis on phosphorylation-driven actions of MLC-2v in adult cardiac muscle, which provide new insights into mechanisms regulating myosin cycling kinetics and human cardiac diseases.

Keywords

myosin light chain-2 (MYL2/MLC-2); cardiac muscle; cardiac disease; contraction; sarcomere; ventricular myosin light chain-2; heart; myosin light chain kinase; cardiac torsion; cardiac function; phosphorylation

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1. Myosin Light Chain-2: Isoforms and Expression Patterns In Striated Muscle

Sarcomeres, the functional units of striated muscle, are composed of a multitude of proteins, precisely organized in a very strict crystalline architecture. Despite the immense similarity in sarcomeric ultrastructure among different vertebrate striated muscles (fast versus slow skeletal muscle and atrial versus ventricular cardiac muscle), there is a very high degree of molecular variability due to the existence of multiple isoforms of each sarcomeric component. The distinct function of each striated muscle type is largely due to the differential expression of sarcomeric protein isoforms (Schiaffino and Reggiani, 1996). Myosin light chain-2 (MYL2/MLC-2) is a major sarcomeric protein in mammalian striated muscle of approximately 19 kDa and 166 amino acids in length and is encoded in human and mouse by the *MYL2* and *Myl2* genes, respectively. Gene structures of *MYL2/Myl2* are publicly available for both human (http://www.ncbi.nlm.nih.gov/gene/4633) and mouse (http://www.ncbi.nlm.nih.gov/gene/17906), which are found on chromosomes 12 and 5, respectively. Protein structures of human and mouse MYL2/MLC-2 are shown in Figure 1. MYL2/MLC-2 belongs to a family of a number of homologous EF-hand Ca^{2+} binding proteins (Grabarek, 2006). The evolutionary lineage of homologous proteins in the EF-hand Ca^{2+} binding superfamily, which includes MYL2/MLC-2 has been previously described and include at least 66 subfamilies that are grouped into two major categories: canonical EF-hands and pseudo EF-hands (Zhou et al., 2006). MYL2/MLC-2 is a member of the CTER (calmoduin, troponin C, essential and regulatory light chains of myosin) subfamily, which encompasses canonical EF-hands that arose from a common four domain precursor, and thus, includes two pairs (forms a globular domain that encompasses side-to-side pairs; Figure 1) of EF-hand calcium binding domains (Kawasaki and Kretsinger, 2014). MYL2/MLC-2 exists as three major isoforms, each encoded by a distinct gene: The fast twitch skeletal isoform designated as MLC-2f, (*Myl2f*; Nudel, et al., 1984), the cardiac ventricular and slow twitch skeletal isoform designated as MLC-2v, (*Myl2*; Henderson, et al., 1988; Kumar, et al., 1986) and the cardiac atrial isoform is designated as MLC-2a, (*Myl2a*; Kubalak, et al., 1994). These three genes are highly homologous with 73% identity and 93% similarity between the primary amino acid sequence of MLC-2v and MLC-2f (Gulick, et al., 1997). A 56% identity and 86% similarity was also observed in the primary amino acid sequence between MLC-2a and MLC-2v (Gulick, et al., 1997).

Despite their high degree of homology, each MLC-2 isoform displays a distinct expression pattern during development. MLC-2f expression is thought to be restricted to skeletal muscles of neonatal and adult mice and rats, although expression in myotomal and extramyotomal muscle-forming regions could also be detected during early embryogenesis (Einat et al., 1987; Faerman and Shani, 1993; Katcoff et al., 1980). On the other hand, MLC-2v is considered one of the earliest markers of ventricular specification during mammalian cardiogenesis (O'Brien, et al., 1993). At E7.5-8.0, MLC-2v is expressed in a restricted zone of the cardiogenic crescent prior to fusion of the bilaterally symmetric progenitors at the mid-line. At E8.0, expression of MLC-2v is restricted within the ventricular segment of the linear heart tube and remains restricted to the ventricular chamber throughout embryonic development into adulthood (O'Brien, et al., 1993; Ross, et al., 1996).

Unlike MLC-2v, which is expressed in both adult ventricular and slow-twitch skeletal muscle, MLC-2a is only expressed in atria (Chen et al., 1998a). During embryogenesis, MLC-2a is expressed somewhat earlier (E7.5) than MLC-2v and is uniformly expressed throughout the heart tube. Its expression is selectively downregulated in the ventricular chamber beginning at E11 and is undetectable in ventricular chambers by E13.5 (Kubalak, et al., 1994). As a result, the disappearance of MLC-2a is considered to be a marker of ventricular maturation (Dyson, et al., 1995). The early ventricular chamber specificity of MLC2v has lead to the creation of a number of novel genetic tools to help researchers drive gene expression to the mouse ventricles *in vivo* (Chen et al., 1998b) as well as used as a selection based method to create human ventricular specific disease models using induced pluripotent stem cell derived cardiomyocytes *in vitro* (Bizy, et al., 2013).

Transgenic approaches have been utilized to dissect the functional differences between the highly homologous MLC-2 isoforms: MLC-2f, MLC-2v, and MLC-2a. Cardiac-specific expression of MLC-2f under the α-myosin heavy chain (MHC) promoter resulted in 100% replacement of MLC-2a protein in the adult atria but only a 53% replacement of MLC-2v protein in adult ventricles (Gulick, et al., 1997). Interestingly, endogenous levels of MLC-2a and MLC-2v mRNA were unaffected in these transgenic lines, indicating that there is no regulatory cross talk between the three MLC-2 isoforms at the mRNA level (Gulick, et al., 1997). Mice with complete replacement of MLC-2a and partial replacement of MLC-2v protein by MLC-2f exhibited a reduction in adult left ventricular contractility and relaxation, as examined in working and non-working isolated heart preparations (Gulick, et al., 1997), demonstrating the non-redundant nature of MLC-2 isoforms since replacement of the skeletal isoform of MLC-2 in the heart contributed to major functional defects in the heart. A limitation to these approaches is the incomplete replacement of isoforms in the adult ventricles presumably due to the existence of endogenous MLC-2v. More importantly, the α-MHC promoter is expressed in both atria and ventricles, thereby making it difficult to interpret the physiological results. Thus, future studies directed at utilizing genetic knock-in strategies to endogenously replace MLC-2v in the MLC-2a locus in mice and vice versa, may help provide further insights into their specialized roles in the heart.

2. Myosin Light Chain-2: The Contractile Protein

Striated muscle contraction occurs when actin-containing thin filaments move past interdigitating myosin-containing thick filaments. The motor in this system is myosin, which transduces chemical energy generated by the hydrolysis of adenosine triphosphate (ATP) into mechanical movement (Huxley and Simmons, 1971). Muscle myosin contains one pair of heavy chains and two pairs of light chains, the essential myosin light chains (MLC-1 or MLC-3) and the regulatory light chain (MLC-2), which is referred to as ventricular myosin light chain-2 (MLC-2v) in cardiac muscle. The three-dimensional structure of myosin indicates that the light chains are arranged in tandem, with MLC-1 and MLC-3 in the amino-terminal half of the neck and MLC-2 in the neck/tail region (Rayment et al., 1993a; Rayment et al., 1993b). Identification of specific interactions between MLC-2 and the cardiac-specific domain of myosin binding protein C further suggested a role for MLC-2 in the myosin tail region, which has exposed binding sites for myosin binding protein C (Ratti et al., 2011). The association between regulatory proteins associated with cardiac myosin,

which include a central role for MLC-2, has been detailed elsewhere (for review see Sheikh et al., 2014).

Genetic loss-of-function studies in mice were amongst the first to demonstrate an essential role for the cardiac isoforms of MLC-2 (MLC-2v and MLC-2a) in cardiac contractile function during early embryogenesis (Table 1). Conventional ablation of MLC-2v in mice resulted in embryonic lethality at E12.5, which was associated with ultrastructural defects in ventricular sarcomere assembly, that included (i) disruptions and disorganization of the normal parallel alignment of the thick and thin filaments, (ii) narrower fiber widths and (iii) larger distances between Z discs and misalignment of Z-band between sarcomeres (Chen et al., 1998a). Global loss of MLC-2a in mice also caused embryonic lethality at E10.5-11.5 and resulted in selective ultrastructural defects in atrial sarcomeric assembly that included (i) absence of myofibrillar organization and (ii) lack of parallel alignment of thick and thin filaments and (iii) lack of appropriate Z disc formation (Huang et al., 2003). Both models also exhibited severe cardiac functional deficits. MLC-2v null hearts manifested into an embryonic form of dilated cardiomyopathy resulting in heart failure with reduced ventricular ejection fraction (Chen et al., 1998a). Interestingly, a compensatory increase in MLC-2a protein levels was observed in MLC-2v deficient ventricles that appeared to incorporate into the thick filament (Chen et al., 1998a). These studies revealed an essential early role for MLC-2v in cardiac contractility as well as ventricular chamber development during cardiogenesis (Chen et al., 1998a). In terms of MLC-2a null mice, the atrial ultrastructural defects manifested into defects in atrial contraction (Huang et al., 2003). Interestingly, striking secondary defects in cardiac morphogenesis (looping) and angiogenesis (extraembryonic and intraembryonic) were also observed in MLC-2a deficient embryos, highlighting that selective loss of embryonic atrial function can have a dramatic impact on cardiac and vasculature development, with cardiac function affecting form (Huang et al., 2003). These studies further shed light on mechanisms (alterations in blood flow) that may impact congenital heart diseases (Huang et al., 2003). More recent studies focused on *tell tale heart*, a mutation identified in zebrafish that encodes the cardiac myosin light chain-2 gene has also revealed a requirement for cardiac MLC-2 in thick filament stabilization (absence of thick filament organization) and contractility in the embryonic zebrafish heart (Rottbauer et al., 2006). Interestingly, zebrafish only contain a single cardiac-specific isoform of MLC-2 and no compensatory increase in another MLC-2 was observed in zMLC-2 deficient settings, demonstrating the requirement of cardiac MLC-2 as a critical contractile protein in the embryonic zebrafish heart (Rottbauer et al., 2006).

3. Ventricular Myosin Light Chain-2: Critical Phosphorylation Sites and Regulators

Phosphorylation of MLC-2 was first discovered in rabbit skeletal muscle (Perrie, et al., 1973; Sweeney et al., 1993) and subsequently in cardiac muscle (Frearson and Perry, 1975; Olsson et al., 2004). In cardiac muscle, the critical phosphorylation sites important for endogenous regulation of MLC-2v phosphorylation *in vivo* are Ser14/Ser15 in the mouse heart and Ser15 in the human heart (Scruggs et al., 2010; Figure 1). Interestingly, compensatory mechanisms exist in the mouse heart to compensate for the loss of one

phosphoryation site, that highlight that both Ser14/Ser15 are required to regulate the levels and functions of endogenous MLC-2v phosphorylation in the heart *in vivo* (Sheikh et al., 2012). The path towards identifying the critical kinase important for phosphorylating MLC-2v has been a long one. Myosin light chain kinase was first purified from rabbit skeletal muscle (Pires and Perry, 1977). The enzyme that phosphorylates MLC-2 is a Ca^{2+}/calmodulin dependent myosin light chain kinase, MLCK (Stull, et al., 1990). In rat ventricle, the basal level of phosphorylated MLC-2v is approximately 30% and increases to about 50% after treadmill exercise (Fitzsimons, et al., 1989). In addition, in skinned cardiac muscle fibers, as in skinned fast skeletal muscle, MLC-2 phosphorylation increased force production at submaximal levels of Ca^{2+} activation (Sweeney and Stull, 1986). It was not until 2007 and 2008 that cardiac myosin light chain kinase (*Mylk3*) was identified as the major regulator of MLC-2v phosphorylation (Chan et al., 2008; Seguchi et al., 2007) as genetic mouse models deficient in cardiac MLCK were sufficient to ablate endogenous MLC-2v phosphorylation levels in the heart *in vivo* (Ding et al., 2010; Warren et al., 2012). Limited studies have focused on identifying the key phosphatases important in regulating MLC-2v dephosphorylation; however, recent studies highlight a contributory role for protein phosphatase 1β (Mizutani et al., 2010). Interestingly, cardiac-specific overexpression of this phosphatase in mice only resulted in a 15% reduction in MLC-2v phosphorylation levels in hearts *in vivo* (Mizutani et al., 2010), suggesting that the main phosphatase and upstream pathways responsible for dephosphorylating MLC-2v remain to be identified. Data from proteomic studies suggest that other post-translational modifications could affect cardiac MYL2/MLC-2 as well as interfere with the phosphorylated states of MYL2/MLC-2, since MLC-2 Ser 15 is also impacted by O-GlcNAacylation (Ramirez-Correa GA et al., 2008). However, further studies are needed to establish the functional role of these modifications on MLC-2v phosphorylation and function.

3.1. Ventricular Myosin Light Chain-2 Phosphorylation: Effects on Cross-Bridge Cycling Kinetics and Cardiac Muscle Contraction

Phosphorylation is a key player in driving a role for MLC-2 in crossbridge cycling kinetics and calcium-dependent muscle contraction. In vertebrate smooth muscle, the regulation of contraction by Ca^{2+} is through the myosin-containing thick filament. Phosphorylation of a single serine residue (Ser19) of MLC-2 causes a conformational change in myosin, triggering interaction of myosin with actin, and activating myosin ATPase activity to result in myofilament movement (Sweeney et al, 1993). In contrast, in vertebrate striated muscle, the regulation of actin-myosin interactions by Ca^{2+} is performed by Ca^{2+} binding to the thin filament regulatory system involving the troponin-tropomyosin complex. In this setting, MLC-2 was thought to have a modulatory effect on muscle contraction (Diffee et al., 2003; Lowey et al., 1993; Sweeney et al., 1993; Szczesna et al., 1996). However, recent studies using computational models and a non-phosphorylatable (Ser14Ala/Ser15Ala) MLC2v knock-in mouse model have revealed a prominent role for MLC-2v in cross-bridge cycling kinetics and cardiac muscle contraction (Sheikh et al., 2012). The mechanisms driving a role for MCL-2v phosphorylation (Ser14/Ser15) in cross-bridge cycling kinetics in cardiac muscle, included increasing myosin lever arm stiffness (results in greater force production by myosin power stroke) and promoting myosin head diffusion (causing myosin heads to move away from the myosin filament backbone and move closer to actin filaments) (Sheikh

et al., 2012). These events altogether slow down myosin kinetics and prolong duty cycle resulting in accumulated myosins being cooperatively recruited to actin binding sites to sustain thin filament activation as a means to fine-tune myofilament Ca^{2+} sensitivity to force (Sheikh et al., 2012). Computational models harboring these mechanisms were sufficient to reproduce data associated with the classic effects of MLC-2v phosphoryation (via MLCK) in adult skinned cardiac myofilaments *in vitro* (Olsson et al., 2004; Stelzer et al., 2006). In addition, computation models harboring loss of these mechanisms were able to reproduce the twitch relaxation defects observed in ex-vivo preparations of papillary muscles from non-phosphorylatable (Ser14Ala/Ser15Ala) MLC2v knock-in mice, which were observed in the absence of changes in Ca^{2+} transients (Sheikh et al., 2012). Thus, these studies further bring to light an important role for MLC-2v phosphorylation in myosin cycling kinetics and a previously unappreciated relationship between muscle tension and Ca^{2+} that is adaptable (Sheikh et al., 2012).

3.2. Ventricular Myosin Light Chain-2 Phosphorylation: Effects on Adult Cardiac Torsion, Function and Disease

Phosphorylation is also a key player in driving a role for MLC-2v in adult cardiac torsion and function (Table 1). In the adult heart, all of the functions of MLC-2v stem from its regulation by phosphorylation, which is uniquely expressed as a spatial gradient (high in epicardium and low in endocardium) across the heart, (Davis et al., 2001; Hidalgo et al., 2006; Sheikh et al., 2012). These expression patterns directly correlated with the expression pattern of its kinase, cardiac myosin light kinase (Warren et al., 2012) and inversely correlated with the expression of its phosphatase, protein phosphatase 1 (Rajashree et al., 2005). The functional relevance of these spatial gradients was only recently revealed as primary defects in cardiac torsion and workload distribution (increased workload in the endocardium versus epicardium) were evident in hearts from a genetic mouse model harboring loss of ML2v phosphorylation (Ser14Ala/Ser15Ala) as well as a genetic mouse model harboring loss of cardiac MLCK at a stage when overt disease was not observed in these models (Sheikh et al., 2012; Warren et al., 2012). Again, computational models harboring loss of myosin cycling mechanisms driving MLC2v phosphorylation were sufficient to reproduce the cardiac torsion defects observed in hearts from non-phosphorylatable (Ser14Ala/Ser15Ala) MLC-2v knock-in mice (Sheikh et al., 2012). These studies further show that spatial differences in MLC-2v phosphorylation and myosin cycling kinetics across the heart not only drive differential mechanics, but that these mechanics impart differential workload distributions across the heart, which are important for driving torsion (Sheikh et al., 2012). A critical role for MLC-2v phosphorylation in adult cardiac function stems from studies performed in a number of genetic mouse models that target loss of MLC-2v phosphorylation and cardiac MLCK as well as overexpression of the myosin phosphatase, protein phosphatase 1, which have been previously comprehensively discussed and summarized elsewhere (Sheikh et al., 2014). Critical to these findings are that MLC-2v dephosphorylation in mice resulted in cardiac dilatation and dysfunction associated with features reminiscent of dilated cardiomyopathy leading to heart failure and premature death (Sheikh et al., 2012; Warren et al., 2012; Mizutani et al., 2012). Interestingly, a cardiac myosin light chain kinase hypomorphic mouse model that resulted in incomplete loss of MLC2v phosphorylation exhibited cardiac hypertrophy, necrosis, fibrosis and cardiac

dysfunction (Ding et al. 2010). These findings altogether highlighted a role for MLC2v phosphorylation as an important contractile protein in the adult heart. These studies further reveal that torsion may be an early manifestation of the loss of MLC2v phosphorylation-mediated mechanisms that drive dilated cardiomyopathy (Sheikh et al., 2012). Support for a role for MLC2v dephosphorylation in dilated cardiomyopathy and heart failure also comes from human studies that have reported MLC2v dephosphorylation in hearts obtained from human patients exhibiting dilated (non-ischemic) cardiomyopathy and heart failure (Morano, 1992; van der Velden et al., 2001; van der Velden et al., 2003a; van der Velden et al., 2003b; Scruggs et al., 2010). In addition, some cases of idiopathic dilated cardiomyopathy in humans are also associated with reduction of MLC2v protein levels in the ventricles due to a specific proteinase mediated cleavage of this light chain (Margossian, et al., 1992). It has been shown by several groups that MLC-2v phosphorylation is also dependent on heart rate and stimulation frequency, suggesting a role for MLC-2v in frequency dependent activation (Lamberts et al, 2007; Varian and Janssen, 2007). In addition, MLC2v phosphorylation has also been shown to increase with stretch in cardiac muscle and recent studies have demonstrated its role in length-dependent activation (Monsasky et al., 2010; Monasky et al, 2013), highlighting a role for MLC-2v phosphorylation in regulatory aspects of cardiac output. Future studies investigating these regulatory mechanisms may also give further insights into the mechanisms regulating the altered myocardial growth response to pressure overload and decline of cardiac function in MLC-2v phosphorylation mutant mice.

MLC2v dephosphorylation has also been reported in human patients carrying a rare form of familial hypertrophic cardiomyopathy (FHC) that impacts cardiac papillary muscle and adjacent ventricular muscle, based on specific MLC2v and MLCK mutations (Szczesna et al., 2001; Davis et al., 2001; Jacques et al., 2008). In addition, other MLC-2v mutations associated with this form of FHC are reported likely to be in close proximity to the Ser15 phosphorylation site (Poetter, et al., 1996). FHC is an autosomal dominantly transmitted disease characterized by ventricular wall thickening (concentric hypertrophy) with myofibrillar disarray (Spirito, et al 1997). These findings are in contrast to findings in genetic mouse models that harbor loss of MLC-2v phosphorylation as the cardiac phenotypes are associated with ventricular wall thinning associated with dilated cardiomyopathy as well as absence of markers associated with FHC such as fibrosis and upregulation of fetal gene markers (Sheikh et al., 2012; Warren et al., 2012; Mizutani et al., 2012). In addition, increased MLC2v phosphorylation is thought to have a cardioprotective role in settings of hypertrophic stress (Huang et al., 2008; Warren et al., 2012), while MLC2v dephosphorylation in mice was shown to result in loss of concentric hypertrophy and an exaggerated switch to eccentric hypertrophy resulting in heart failure in settings of cardiac (pathological and physiological) hypertrophic stress (Sheikh et al., 2012; Warren et al., 2012). Mutations in the *MYL2* gene have been associated with cardiomyopathies and been extensively studied (Figure 1). These mutations range from the recently association with dilated cardiomyopathy (D94A) to the rare form of familial hypertrophic cardiomyopathy (A13T, F18L, E22K, N47K, R58Q, P95A, K104E, D166V) (Abraham et al., 2009; Farman et al., 2014; Greenberg et al., 2009; Huang et al., 2014; Huang et al., 2015; Muthu et al., 2012; Szczesna-Cordary et al., 2004). Interestingly, ex-vivo expression

of MLCK (and restoration of MLC2v phosphorylation) could rescue the cardiac contractile defects but not force deficits in hearts of a transgenic mouse model harboring the FHC-associated MLC-2v D166V mutation (impacts Ca^{2+} binding affinity) (Muthu et al., 2012), demonstrating the impact of restoring myosin cycling kinetics in the setting of FHC. Recent studies have also revealed novel mechanisms that are influenced by MLCK-mediated pathways, such as ubiquitin-mediated protein degradation pathways (Warren et al., 2012), which may also contribute to the effects of MLC-2v dephosphorylation in the heart, that have yet to be fully explored in FHC settings. Thus, future studies focused on unlocking the mechanisms driving the effects of MLC2v phosphorylation in hypertrophic stretch activated settings may also help explain the variable and rare phenotypes observed in FHC.

4. Conclusions

From its initial discovery in 1969 (Weeds, 1969), MLC-2 has emerged as an established early cardiac developmental marker, with data from genetic mouse models cementing its role as an essential contractile protein in both the embryonic and adult heart. Although previously thought to have a modulatory role in cardiac muscle, new data from computational models and genetic mouse models deficient in the phosphorylatable form of MLC-2v have also elucidated mechanisms driving its role in myosin cycling kinetics and calcium-dependent contraction in cardiac muscle (Table 2). Importantly, recent studies have also demonstrated that the transmural gradient of MLC-2v phosphorylation plays a critical role in adult cardiac torsion, function and diseases, including dilated cardiomyopathy and heart failure (Table 2). However, mechanisms by which MLC-2v mutations found in a rare form of FHC result in disease manifestation are not clear, but may involve the role of MLC-2v phosphorylation in the hypertrophic stretch response. Greater understanding of the role of MLC-2v phosphorylation in cardiac muscle will help fuel therapeutic approaches directed at restoring MLC2v phosphorylation, which appear to have a cardioprotective role in the setting of hypertrophic stress.

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Abbreviations

ATP	Adenosine triphosphate
Ca^{2+}	Calcium
CTER	Calmoduin, troponin C, essential and regulatory light chains of myosin
E	Embryonic day
FHC	Familial hypertrophic cardiomyopathy

MHC	Myosin heavy chain
MLC-1	Myosin light chain-1
MYL2/MLC-2	Myosin light chain-2
MLC-2a	Atrial myosin light chain-2
MLC-2f	Fast-twitch skeletal myosin light chain-2
MLC-2v	Ventricular myosin light chain-2 and slow twitch skeletal myosin light chain-2
MLC-3	Myosin light chain-3
MLCK	Myosin light chain kinase
Ser15	Serine 15 phosphorylation site
Ser14/Ser15	Serine 14 and 15 phosphorylation sites
Ser14Ala/Ser15Ala	Serine 14 and 15 phosphorylation mutations to Alanine 14 and 15
Ser19	Serine 19 phosphorylation site
zMLC-2	Zebrafish myosin light chain-2

Highlights

- MYL2 is sarcomeric protein and member of the EF-hand calcium-binding protein family
- MYL2 plays an important role in embryonic heart muscle structure and function.
- In adults, MYL2 phosphorylation regulates cardiac myosin cycling kinetics, torsion and function.
- Critical phosphorylation sites include: Ser14/Ser15 in mice and Ser15 in humans.
- MLC-2v dephosphorylation has been implicated in dilated cardiomyopathy and heart failure.

Figure 1.

Human and mouse MLC2v protein structures illustrating the location of EF-hand, calcium binding motifs and the sites of dominant missense mutations found in patients with dilated (D94A; red) and hypertrophic cardiomyopathy (A13T, F18L, E22K, N47K, R58Q, P95A and D166V; black). The location of key phosphorylation sites (S14 and S15) implicated in cardiac function and disease are also denoted.

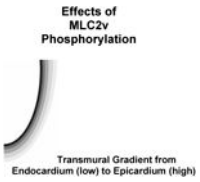
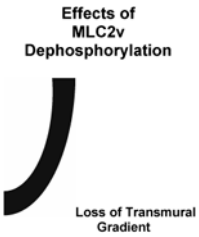
Table 1

Summary of Genetic Models Demonstrating An Essential Role for Myosin Light Chain-2 in the Embryonic and Adult Heart

Genetic Model	Phenotype	Reference
Conventional ablation of mouse Ventricular Myosin Light Chain-2	Embryonic lethality at E12.5 associated with ultrastructural defects in ventricular sarcomere assembly resulting in a dilated cardiomyopathy and heart failure associated with reduced ejection fraction.	Chen et al., 1998b
Conventional ablation of mouse Atrial Myosin Light Chain-2	Embryonic lethality at E10.5-11.5 associated with ultrastructural defects in atrial sarcomere assembly resulting in reduced atrial contraction and leading to secondary effects in cardiac morphogenesis (looping) and vasculature (angiogenesis) development	Huang et al., 2003
Mutation in a zebrafish Myosin Light Chain-2 (*tell tale heart*)	Embryonic lethality associated with absence of thick and thin filament assembly as well as contractile defects (silent atria and ventricles, loss of fractional shortening) resulting in pericardial edema	Rottbauer et al., 2006
Ventricular Myosin Light Chain-2 Phosphorylation Mutant (Ser14Ala/ Ser15Ala) knock-in mouse model	Adult lethality associated with early defects in cardiac twitch relaxation and torsion leading to dilated cardiomyopathy, heart failure and premature death	Sheikh et al., 2012
Cardiac myosin light chain kinase hypomorphic mouse	Adult phenotype with exaggerated cardiac hypertrophy, necrosis, fibrosis and decreased cardiac function	Ding et al., 2010
Conventional ablation of mouse cardiac Myosin Light Chain Kinase	Adult phenotype associated with early defects in cardiac torsion leading to dilated cardiomyopathy	Warren et al., 2012
Cardiac specific overexpression of mouse protein phosphatase 1-b	Adult phenotype associated with dilated cardiomyopathy	Mizutani et al., 2012

Table 2

Summary of the Impact of MLC2v phosphorylation status on cardiac muscle biology and physiology

		
Cross-Bridge Cycling Kinetics	- Cardiac muscle contraction - Increased myosin lever arm stiffness (greater force production by myosin power stroke) - Promote myosin head diffusion (increase myosin heads moving to actin filament) - These events culminate in slowing down myosin cycling kinetics to prolong duty cycle	-Decreased myosin lever arm stiffness & myosin head diffusion to accelerate myosin cycling kinetics to shorten duty cycle
Skinned Cardiac Muscle	- Increase in maximum contraction force (maximum tension) due to sustained myofilament thin filament activation - Increase in calcium sensitivity of contraction to force (*pCa50*) - No detectable changes in the kinetics of force redevelopment following stretch	-Decrease in maximum tension & calcium sensitivity of contraction to force
Whole Cardiac Muscle	-Decreased twitch relaxation	-Accelerated twitch relaxation in the absence of changes in calcium cycling kinetics
Whole Heart	-Increase in cardiac torsion to maintain low subendocardial workload -Maintenance of cardiac structure & function -Myocardial growth response to pressure overload (concentric hypertrophy) - Regulatory aspects of cardiac output (frequency and length dependent activation)	-Loss of cardiac torsion, which was associated with increased subendocardial myofiber workload & disease vulnerability -Dilated cardiomyopathy associated with Z disc widening & heart failure -Loss of myocardial growth response to pressure overload (eccentric hypertrophy)