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Review

Muscle LIM Protein: Master regulator of cardiac and skeletal muscle functions

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ABSTRACT

Muscle LIM Protein (MLP) has emerged as a key regulator of striated muscle physiology and pathophysiology. Mutations in cysteine and glycine-rich protein 3 (*CSRP3*), the gene encoding MLP, are causative of human cardiomyopathies, whereas altered expression patterns are observed in human failing heart and skeletal myopathies. In vitro and in vivo evidences reveal a complex and diverse functional role of MLP in striated muscle, which is determined by its multiple interacting partners and subcellular distribution. Experimental evidence suggests that MLP is implicated in both myogenic differentiation and myocyte cytoarchitecture, although the full spectrum of its intracellular roles still unfolds.

Contents

1. Introduction

The cysteine and glycine-rich protein 3 (*CSRP3* or *CRP3*) gene is a member of cysteine-rich protein (CRP) family that consists of *CRP1*, *CRP2* and *CSRP3* (Weiskirchen et al., 1995). CRP1 and CRP2 are prominent in smooth muscle, whereas CSRP3 is expressed in striated muscle (Weiskirchen et al., 1995; Louis et al., 1997). MLP, the protein encoded by *CSRP3*, belongs to the LIM-only domain family, a large protein family with diverse functional roles, including transcriptional regulation, cell fate determination, cell adhesion and motility, cytoskeleton organization and signal transduction (Schmeichel and Beckerle, 1997; Kadrmas and Beckerle, 2004; Zheng and Zhao, 2007). Its diverse functional roles have a significant impact on cardiac muscle physiology and pathology and skeletal muscle physiology and pathology.

Abbreviations: MLP, Muscle LIM Protein; CSRP3, cysteine and glycine-rich protein 3; CRP3, cysteine-rich protein; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; CFL2, cofilin-2; MRF4, myogenic regulatory factor 4; PLN, phospholamban.

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Since the identification of MLP, 20 years ago (Arber et al., 1994), a multitude of studies have focused on delineating its functional significance in striated muscle, with exciting findings to date. These investigations were intensified once MLP was implicated in muscle pathogenesis. Mutations in the *CSRP3* gene have been directly associated with dilated (DCM) and hypertrophic (HCM) cardiomyopathies while the MLP protein levels appear to be significantly altered in human failing hearts and various skeletal myopathies (Knoll et al., 2002a; Gehmlich et al., 2008). Several in vitro and in vivo approaches have been used to decipher the MLP's multifaceted role in pathological settings (Knoll et al., 2002a; Gehmlich et al., 2008). Herein, we discuss the intricate involvement of MLP in striated muscle function and disease, the emerging hypotheses, and open questions.

2. *CSRP3* gene structure and transcription

CSRP3 was first identified in 1994 during a rat cDNA library screen for genes regulating muscle gene expression and neuromuscular synapses formation (Arber et al., 1994). Northern blot analysis demonstrated its predominant expression in striated muscles (Arber et al., 1994). In 1995, the human *CSRP3* gene was mapped to chromosome 11p15.1 and subsequently isolated from a human cardiac cDNA library (Fung et al., 1995, 1996). The human gene spans a 20 kb genomic region, producing a 0.8 kb transcript that is organized in 6 exons (Fung et al., 1996; Knoll et al., 2002b). We recently reported the discovery of the first splice variant of *CSRP3*, originating from alternative splicing of exons 3 and 4 (Vafiadaki et al., 2014). This novel MLP isoform, designated MLP-b, was demonstrated to exhibit distinct expression and functional roles to the full length MLP, revealing the complexity of MLP expression and intracellular roles.

CSRP3 expression was found to be largely determined by an E-box sequence at the position −186 to −180 within the *CSRP3* promoter region (Ji et al., 2009). Synergistic binding of the transcription factors myogenin and myocyte enhancer factor 2C (MEF2C) occurs at the E-box promoter region, and leads to transcriptional activation of *CSRP3*. Additional cis-acting elements such as the binding sites for AP1, Jun/Fos are suggested to be present in the promoter (Ji et al., 2009); however, their role in regulating the *CSRP3* promoter is unclear.

3. MLP structure and physicochemical properties

MLP is a relatively small protein of 194 amino acids that is specifically expressed in skeletal and cardiac muscles. Structurally, it belongs to a family of proteins that harbor one or more LIM domains. The name of this protein domain was derived from the initial letters of Lin-11, Isl1 and Mec-3 that represent the first three members of this protein family to be identified (Schmeichel and Beckerle, 1997; Kadrmas and Beckerle, 2004). In general, LIM domains comprise of approximately 50–60 amino acids and share two characteristic zinc finger domains. These zinc fingers contain 8 highly conserved cysteine and histidine residues at specific positions, which coordinately bind two zinc ions (Zheng and Zhao, 2007). The overall consensus sequence of a LIM domain is $CX_2CX_{16\_23}HX_2CX_2CX_2CX_{16\_21}CX_2$(C/H/D), where X denotes any amino acid (Schmeichel and Beckerle, 1997; Kadrmas and Beckerle, 2004). LIM domains may be found throughout the length of a protein. They are believed to act as a modular protein-binding interface that mediates diverse protein interactions and facilitates macromolecular complex formation, thus enabling LIM domain proteins to participate in a broad range of biological functions (Schmeichel and Beckerle, 1994; Arber and Caroni, 1996; Weiskirchen and Gunther, 2003; Kadrmas and Beckerle, 2004).

MLP contains two LIM domains (LIM1 and LIM2), each surrounded by glycine-rich repeat regions, and the two separated by more than 50 residues (Weiskirchen et al., 1995). The presence of these glycine-rich repeats is characteristic of all CRP family members and distinguishes them from other LIM-only containing proteins (Weiskirchen and Gunther, 2003). While the structural analysis of CRP1 and CRP2 proteins was completed several years ago (Perez-Alvarado et al., 1994; Konrat et al., 1997; Kontaxis et al., 1998; Yao et al., 1999), it was only recently that this information became available for MLP (Schallus et al., 2007, 2009). Using nuclear magnetic resonance spectroscopy, it was deduced that the two MLP LIM domains act as independent units and that the adjacent linker region is fully flexible (Schallus et al., 2007, 2009). These structural characteristics suggest that LIM domains could act as adaptors facilitating the formation of macromolecular complexes. Indeed, the two MLP LIM domains mediate interactions with a vast number of different proteins at different subcellular locations, including the cytoplasm and the nucleus (see the MLP protein interactions and localization section) (Buyandelger et al., 2011b).

An emerging concept is this of MLP oligomerization. Using biochemical subcellular fractionation and immunocytochemistry techniques it was shown that MLP forms dimers, trimers and tetramers in myocytes (Boateng et al., 2007, 2009). The oligomerization potential of MLP is believed to be critical for its cellular localization and function, with direct implications in muscle physiology.

4. MLP post-translational modifications

Post-translational modifications represent key determinants of protein function. The precise effect of post-translational modifications on MLP is to date largely unknown. The only available experimental evidence shows the acetylation/deacetylation of MLP at a lysine residue at position 69 (K69), by acetyltransferase (PCAF) and histone deacetylase 4 (HDAC4), respectively (Gupta et al., 2008). Since the K69 residue is within the predicted nuclear localization signal of MLP (Fung et al., 1996), it was proposed that acetylation of K69 may influence MLP nucleocytoplasmic shuttling. K69 acetylation was further associated with calcium sensitivity and myofilament contractility (Gupta et al., 2008). These findings reveal the emerging role of reversible acetylation in the regulation of muscle contraction.

In addition to acetylation, it has been postulated that MLP may undergo phosphorylation or sumoylation, which could in turn modulate its localization and/or function (Buyandelger et al., 2011b). Bioinformatical analysis predicts the existence of several putative phosphorylation sites on MLP (Fig. 1); however, no evidence on the occurrence of MLP phosphorylation has been published to date so experimental confirmation and functional characterization is pending.

5. MLP protein interactions and localization

Determining the localization and binding partners of MLP has been an integral part of the quest for its spectrum of intracellular roles. This has led to an increasingly large list of interacting proteins with diverse functional properties, as well as variable subcellular localizations within muscle cells. In particular, in the cytoplasm MLP has been detected at the level of the sarcomeres (the basic muscle contractile unit, delimited by Z-disks and an M-band in the center), intercalated disks (located at the bipolar ends of cardiomyocytes, inter-connecting cardiomyocytes, enabling the synchronized contraction of cardiac tissue), costameres (a critical component of striated muscle morphology connecting the sarcomeres to the sarcolemma), and even the cell membrane. At the sarcomeres MLP interacts with the Z-disk proteins telethonin (T-cap), α-actinin, cofilin-2 (CFL2), calcineurin, HDAC4, as well as MLP and MLP-b (Louis et al., 1997; Zolk et al., 2000; Knoll et al., 2002b; Heineke et al., 2005; Gupta et al., 2008; Papalouka et al., 2009; Vafiadaki et al., 2014). Although LIM domains are major protein-binding interfaces, we have demonstrated that the MLP inter-LIM region, containing a glycine-rich repeat, can itself serve as the minimal binding region, with proteins such as CFL2 and the MLP-b isoform (Papalouka et al., 2009; Vafiadaki et al., 2014). The functional implications of these interactions are discussed in the next section. M-band distribution of MLP has also been described, although the binding partners mediating this