Muscle LIM protein in heart failure

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Z-line protein have important structural functions. Recent publications point to additional, previously unexpected functions and new views are now emerging. Z-line proteins are involved in important intra- and intercellular signalling pathways. They translocate into the nucleus, they interact with a variety of signalling molecules including kinases and transcription factors, and they have the ability to form macromolecular protein complexes indicating furthermore their multifuntionality. The muscle LIM protein (MLP) is muscle specific, and is expressed and located at the z-line. MLP's physiological role at the z-line and in the nucleus may be better understood by precise investigations of specific mutations in specific domains of this protein.

Key Words: *Cardiomyopathy; Heart failure; Muscle LIM protein; Z-line proteins*

The muscle LIM protein (MLP) belongs to a structurally related family of proteins harbouring either one or several different LIM domains (LIM is itself an acronym resulting from the initials of the first three members of this increasingly growing family of proteins: lin-11, islet-1, mec-3). The LIM domain, characterized by the cysteine-rich consensus CX2CX16-23HX2CX2CX2CX16-21 CX2-3(C/H/D), is a specific metal-binding structure that consists of two distinct zinc-binding subdomains. To date more than 60 members of this protein family are known (1).

LIM proteins are divided into mainly three groups: (a) LIM-only proteins, consisting of one or several LIM domains; (b) LIM functional domain proteins (such as LIM kinases); and (c) LIM homeodomain proteins, consisting of one or several LIM domains connected to a homeodomain. MLP belongs to the first group and consists of two LIM domains, each followed by a glycine-rich domain. The next vertebrate relatives are the cysteine-rich proteins (CRP1 and CRP2). CRP1 is expressed in vascular and visceral smooth muscle whereas CRP2 is prominent in vascular smooth muscle. MLP, also referred to as CRP3, is expressed in striated muscle only. LIM domain proteins are thought to function as adapters, facilitating macromolecular complexes (2). It is very likely then that MLP also creates macromolecular protein networks. It is important to mention in this context actinin-binding LIM protein (ALP), another cardiac LIM protein, localized at the intercalated disc. It interacts with alpha-actinin and gamma-catenin, the latter being associated with arrythmogenic right ventricular cardiomyopathy in humans. Interestingly, ALP knockout animals develop a right ventricular cardiomyopathy (3). Another cardiac LIM protein is cypher, located at the z-line and, in a mouse knockout model, associated with a severe congenital myopathy. This protein seems not to be required for sarcomerogenesis or z-line assembly, but it is required for the maintenance of the z-line during muscle function (4).

MLP was cloned in 1994 (5) and, because of its potential to induce myogenic differentiation, described as a novel essential regulator of myogenesis. Targeted ablation of the MLP gene in a mouse model led to the development of dilated cardiomyopathy and thus illustrated in the first genetically manipulated organism with this phenotype (6).

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Surprisingly, generation of MLP-phosholamban double knockout animals results in lifelong and complete rescue from the onset of dilated cardiomyopathy (ie, super-rescue) (7). The protein was initially found to be present not only in the actin-based cytoskeleton but also in the nucleus, and a nuclear localization-like sequence, located after the first LIM domain (YGPKG), was described (8). As a consequence, in one study (9) MLP interaction with myoD, MRF 4 and myogenin was suggested and hence a function for MLP as a cofactor of transcription proposed. In addition to the abovementioned nuclear proteins, only a few other MLP interacting proteins are known: actin (10), alpha-actinin (11), beta-spectrin (12) and N-RAP (13).

In an attempt to study right ventricular heart failure induced by pulmonary hypertension in rats, Ecarnot-Laubriet et al (14) observed a marked 50% downregulation of MLP transcripts as well as a loss of MLP immunoreactivity. Notably, MLP nuclear relocalization following hemodynamic overload was presented (14).

In another approach, human-explanted, idiopathic-dilated cardiomyopathic, ischemic cardiomyopathic and nonfailing human donor hearts were analyzed for their MLP expression using northern blot and immunohistochemistry. Surprisingly, there was no difference in MLP mRNA expression between the groups, but there was a 50% significant downregulation of MLP protein in the idiopathic-dilated cardiomyopathic hearts (15).

Using chronic low frequency stimulation in a rat model, Schneider et al (16) showed a significant MLP overexpression. This expression was not due to detectable signs of myogenesis or fibre repair or regeneration and thus was most likely due to biomechanical stress caused by the stimulation. In other words, enhanced contractile activity promotes MLP expression.

Given that MLP-deficient mice develop a form of dilated cardiomyopathy with many of the major features present in humans, downregulation of MLP in human idiopathic dilated cardiomyopathy might be of pivotal importance, implying a basic mechanism underlying the human condition. This point of view is further supported by the contributions of Ecarnot-Laubriet et al (14) and Schneider et al (16), demonstrating a relationship between contractility and MLP expression. In addition, Ehler et al (13) reported an MLP downregulation in another type of DCM, the tropomodulin overexpression transgenic mouse. Taken together, MLP downregulation seems to be a conserved feature in different settings of heart failure. In contrast, the underlying mechanisms remain to be elucidated. Given the present data, a transcriptional mechanism is also not likely in human conditions of ischemic and dilated cardiomyopathy (15); in other experimental settings a transcriptional mechanism cannot be excluded because in the work of Schneider et al (16) and of Ecarnot-Laubriet et al (14), correlation between the MLP mRNA and protein expression was presented. Only in humans is there a dissociation between the mRNA and protein content. However, the result, MLP downregulation in human ischemic and dilated cardiomyopathy, is an important finding.

Rarefication of sarcomeres and an increase in nonmyofibrillar space is well known in cardiomyopathies (17). It will be interesting to correlate the MLP protein contents to the actual presence of myocardial tissue.

It is important to analyze MLP expression in other forms of human heart disease, ie, hypertropic cardiomyopathy, restrictive cardiomyopathy and congenital cardiomyopathy. Much more research should be performed to find out whether the observed MLP downregulation in several different conditions of heart failure is a primary event or whether it is only a secondary effect as a consequence of the deleterious physiological situation. Most interesting of all will be whether the normalization of cardiac function, after therapy, restores MLP expression, or whether the restoration of MLP expression improves cardiac function.

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