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MyBP-C in cardiac conditions and its potential use as novel biomarker: a review

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Abstract:

Being cardiac specific, Myosin Binding Protein C (MyBP-C) is becoming a protein of interest clinically. Studying MyBP-C from different aspects enable us to better understand its involvement in certain cardiac conditions such as cardiomyopathies (HCM and DCM) and heart failure. By studying its release, kinetics and clearance from the circulation and by comparing to other conventional biomarkers, it has been reported that MyBP-C can be used as a novel biomarker for AMI. Moreover, studying the genetics and their pathogenicity has opened the ideas for potential therapeutic strategies.

Key words: MyBP-C, Cardiac Biomarker, HCM, DCM, myocardial infarction, heart failure

INTRODUCTION

MyBP-C is a cardiac protein encoded by MyBPC3 gene. It is different from other isoforms MYBPC1 and MYBPC2, which are expressed in slow and fast skeletal muscle respectively.

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MyBP-C is structurally different from the other isoforms by possessing an additional immunoglobulin-like domain at N terminus (C0 domain), having multiple phosphorylation sites between C1 and C2 domains (M motif) and having C5 domain. Recently, MyBP-C has been a protein of interest for many researchers not only for its functional importance within the sarcomere and during the cardiac cycle, but also as novel biomarker for cardiac dysfunction and diseases as well as for developing therapeutic strategies.

Our aim in this review is to emphasize the importance of MyBP-C as an important regulatory protein of the sarcomere and how altered physiological function of c-MyBP-C can be involved in the pathogenesis of HCM, DCM and impaired systolic and diastolic function. Moreover, several studies have shown that plasma MyBP-C can be used as potential biomarkers in various cardiac conditions. By understanding the importance and function of MyBP-C as a regulatory protein, this can help in development of therapy for cardiac conditions. Also by studying the release, kinetics and clearance of plasma level MyBP-C, we can compare it to other conventional biomarkers whether it could be used as a novel biomarker in certain cardiac conditions.

STRUCTURE AND FUNCTION OF cMyBP-C

MyBP-C is a cardiac protein specific to the heart whose importance is to stabilize the sarcomere and help in crossbridge formation through regulation of actomyosin. [1,2] MyBP-C comprises of a complex structure including 12 domains, namely C0-C10 from the N terminus and the M domain. The C-terminal (C10) binds the thick myosin filament backbone, and the N-terminal domains extend radially from the thick filament. [3]The N-terminal domains are positioned in such a way that they can bind to actin filaments or to myosin S2 domain. The "M-domain", also known as the motif, are long

polypeptide linkers found between C1 and C2 domains. This M-domain contains multiple phosphorylation sites, mainly four highly conserved serines (S273, S282, S302, and S307).[4,5] ß-Adrenergic–stimulated phosphorylation of these serines is believed to regulate crossbridge formation through actomyosin and thus enhance cardiac contractility[6,7], and a high level of phosphorylation appears to be important for normal cardiac cycle, whereas inability to phosphorylate efficiently has been associated with heart failure.[8]

ROLE OF MyBP-C IN CONTRACTILE CYCLE

The sarcomere is the basic contractile unit within a myocyte, which is composed of myofibrils. Each myofibril consists of thin and thick filaments. The thin filament is composed of actin protein and thick filament consists of myosin protein. Sarcomere refers to the unit from one Z band to the next whose resting length is about 1.8-2.4 mm. Many regulatory elements within the sarcomere such as tropomyosin, troponin, titin, myosin binding protein C among others, work together to ensure the normal contractile function.

The large level of intracellular calcium ions released from the sarcoplasmic reticulum binds to TnC of Troponin complex and TnI is inhibited. Mainly the C1 component of the N-terminal domain of the cMyBP-C bind to induce a conformational change and shift the tropomyosin complex[9], thus activating the thin filament into an open structural state and exposes the binding site of actin. Myosin heads interact with active sites on actin filaments in an ATP-dependent reaction. Hydrolysis of ATP on the myosin induces crossbridge formation between myosin head and active site on actin.[10] The strength of contraction is said to be proportional to the number of cross bridges formed.[7]

β-ADRENERGIC –STIMULATED PHOSPHORYLATION OF cMyBP-C

The two important cardiac proteins namely C-TnI and cMyBP-C, being phosphorylated following ß-adrenergic stimulation, have crucial role in modulating ventricular contraction and relaxation in order to meet the circulatory demand.[7,11]   In response to ß-adrenergic stimulation, phosphorylation of MyBP-C is enhanced mainly at the M motif via protein kinase A, resulting in positive inotropic cardiac effects. Studies have show that there is high level of phosphorylation in normal heart as opposed to failing heart such as in HCM condition, whereby most of MyBP-C are unphosphorylated.[12]

EFFECTS OF PHOSPHORYLATION OF MyBP-C ON SYSTOLIC FUNCTION

During systolic phase of the cardiac cycle, there is pressure development in the ventricle. The rate at which pressure develops is largely dependent on the rate of cross bridges attachment and the availability of the myosin-binding site on the thin filament.[13] The force developed within the myocardium relies on the rate at which cross bridges are recruited.[7,14] PKA dependent phosphorylation of MyBP-C is crucial to increase the rate of formation and recruitment of cross bridges and activation of thin filament. This in turn is involved in the acceleration of the rate of force developed within the myocardium and well as contribute to the rate of pressure development as confirmed by previous report.[15] During systole, the ventricular pressure continues to rise up to a point where the pressure in the ventricle exceeds that in the aorta, thereby the aortic valve opens and blood is pumped out into the peripheral circulation. During that time, the ventricular pressure continues to persist but at a much lower rate than during IVC until a maximum ventricular pressure is achieved.

It is ultimately the number of force-generating cross bridges attached to the thin filament that determines the peak pressure achieved.[16] An increase in the number of force generating cross bridges would increase the rate and peak pressure developed during systole and a decrease of force generating cross bridges would be expected to decrease the rate and peak pressure development. This speculation is indeed observed in previous studies conducted. A decrease in maximal peak pressure has been observed in MyBP-C phospho-ablated mice in response to acute dobutamine administration or to increase pacing frequency.[17] This clearly suggests that MyBP-C dephosphorylation decreases the recruitment of the force generating cross bridges and result in a decreased maximal pressure generated and blood ejected during systole. Phosphorylation is increased in response to adrenergic stimulation.[18] Thus, the heart ability to respond to adrenergic stimulation would be severely impaired as a result of MyBP-C dephosphorylation by not able to increase the cardiac output in conditions of increased circulatory demand.

EFFECTS OF PHOSPHORYLATION OF MyBP-C ON DIASTOLIC FUNCTION

Ventricular relaxation is initiated by the removal of calcium ions from the cytoplasm. The rate of ventricular pressure relaxation is however mediated by other independent processes [19] such as the rate of deactivation of thin filament and the rate of cross bridges detachment. Studies have shown that the phosphorylation of TnI and MyBP-C by PKA decreases the TnC affinity towards Calcium ions and accelerates the dissociation of crossbridges, hence enhancing relaxation.[20,21] The time required to reach maximum relaxation is increased if ever MyBP-C cannot phosphorylates properly.[7] This can be explained by the slow deactivation of crossbridges from the thin filament. Studies have reported that the effect of MyBP-C

phosphorylation increases the rate of crossbridges detachment, hence enabling proper relaxation.[22] Dephosphorylation of MyBP-C can lead to diastolic dysfunction as seen in HCM.[23] Thus, MyBP-C dephosphorylation will ultimately lead to slow deactivation of thin filament and impaired rate of detachment of crossbridges from the thin filament. As a result of this, ventricular relaxation will be both affected and prolonged. We can conclude that MyBP-C phosphorylation accelerates rate of cross-bridges detachment therefore promoting relaxation for proper diastolic function.

MYBPC3 AND HYPERTROPHIC CARDIOMYOPATHY

MYCBPC3 mutation is the most frequently mutated gene in HCM. [24] About 40% of patients who have been clinically diagnosed with HCM have been attributed to have MyBPC3 mutations.[25] HCM has a prevalence of 1:1500 in young adults[26] and is the most common cause of sudden death among the young.[27] It is an autosomal dominant myocardial disease characterized by left ventricular hypertrophy, myocardial disarray and diastolic dysfunction.[28] More than 350 individual MYBPC3 mutations have been identified causing HCM.[29] Different type of mutation can occur in MyBP-C such as missence mutation (most common type), insertion or deletion, premature mutation, frameshift mutation, intronic mutation among others. Different mutation can occur at different domains of the MyBP-C and thus cause different structural or dysfunction of the MyBP-C protein. For instance, a Japanese study showed that left ventricular remodeling of HCM is cause by a frameshift deletion mutation in MyBP-C.[30] Another study showed that a missense mutation on MyBP-C, which would alter a one amino acid in the MyBP-C protein, was responsible for HCM with left ventricular dysfunction and dilation in elderly.[31]

THE ROLE OF MyBP-C IN DCM

MyBP-C, similarly to cTnI, may be involved in the pathogenesis of DCM though eliciting an autoimmune response, resulting in production of auto-antibodies.[32] Fragment release of C0C1 following proteolysis of MyBP-C post-MI elicits production of auto-antibodies. These auto-antibodies are believed to play in role in the onset of autoimmune myocarditis which can ultimately progress to DCM and heart failure.[33] Studies have attributed the presence of cardiac protein auto-antibodies to onset of autoimmune myocarditis and DCM.[34] Presence of MyBP-C-reactive AAbs in the sera of DCM patients was observed in previous report. [35

MyBP-C AS A DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN HEART FAILURE

Circulating MyBP-C turns out to have 100% sensitivity and 96%specificity at cutoff value of 45 ng/ml and be a good diagnostic biomarker for heart failure.[36] Other studies also confirmed elevated level of MyBP-C in heart failure [37] The exact mechanism of MyBP-C release in heart failure is still unknown. Cardiac stress can impair MyBP-C phosphorylation at the M motif. It has been shown that decreased phosphorylation would ultimately lead to impaired cardiac contractility and has been associated with failing heart.[38] Moreover, a 152 ng/ml cutoff value of MyBP-C has a 90% sensitivity and 93% specificity as a biomarker of prognosis in heart failure patient.[36] Failure to decrease the level of MyBP-C with prompt therapy is linked with poor prognosis.[36] Hence, MyBP-C has both diagnostic and prognostic importance clinically as a biomarker of heart failure.

APPLICATION OF MyBP-C AS A NOVEL BIOMARKER IN MYOCARDIAL INJURY

Cardiac troponin (cTn) has become an important biomarker and is used for diagnosis of AMI. Myocardial injury requires quick and prompt attention and researchers are still looking for a better and absolute biomarker than cTn. MyBP-C is ranked 19th out of 2300 in term of the most voluminous cardiac protein quantified, twice as much as cTnI and cTnT.[39] Following MI, catalytic cleavage of C0-C1 fragment of the N terminal is induced and this cleaved fragment, which is released in the circulation, can be used as potential marker.[40] Studies have reported MyBP-C fragments in mouse sera[41] as well as in patients following myocardial infarction[40,42,43]. Studies have even gone farther to understand the kinetics of release and clearance compared to cTnT. Interestingly, it was found that MyBP-C, as a biomarker, rises and clears more rapidly as compared to cTnT.[43,44] With these characteristics and different kinetics as compared to cTnT, MyBP-C is a biomarker of interest for many researchers and a potential biomarker for myocardial injury. Release of MyBP-C fragments from catalytic cleavage after myocardial infarction has been linked with contractile dysfunction[32], but the exact underlying mechanism is still not fully understood. It is speculated that increased levels of calcium ions in ischemic conditions or early reperfusion leads to the activation of calpain enzyme.[45] The latter is responsible for the breakdown of proteins including regulatory proteins during ischemic attack.[46] Dephosphorylation of MyBP-C make it more prone to proteolysis by calpains, inducing release of C0C1 fragments.

A BETTER UNDERSTANDING OF MyBP-C FOR A THERAPEUTIC POTENTIAL IN TREATMENT OF CARDIAC CONDITIONS

A prompt understanding of MyBP-C in term of structure, genetics and functions is of primordial importance and can be helpful to develop therapeutic potential strategies in treatment of HCM, DCM, heart failure, AMI and other related cardiac conditions. It is clear that MyBP-C is involved in the pathogenesis of heart failure due to markedly reduced phosphorylation.[7] Therapeutic strategies can be developed to focus on enhancing phosphorylation of MyBP-C as a potential treatment in heart failure. This also can make us re-evaluate our treatment plan concerning heart failure. We have seen above how ß-Adrenergic –stimulation is important for phosphorylation of MyBP-C.[7] As already discussed above, hypophosphorylation is associated with failing heart. ß-Adrenergic blockers are commonly prescribed drugs for HFpEF. The main cause of HFpEF is thought to be impaired diastolic function.[47] Based on this logic, using ß-Adrenergic blockers would exacerbate hypophosphorylation and thus affecting more the diastolic dysfunction. On the other hand, finding ways that favors the phosphorylation of MyBP-C would be a novel therapy for HFpEF.

MyBP-C is also involved in the pathogenesis of HCM in multiple ways ranging from mutations to environmental risk factors.[48] An up regulation in the expression of MyBP-C is observed in HCM.[49] There is even a correlation between MyBP-C and degree of hypertrophy of the heart.[50] Based on a mouse model study, ablation of MyBPC3 made the mice resistant to develop HCM.[51] This could mean that knocking down of MyBPC3 may be a therapeutic potential method to treat or prevent HCM. If an increase expression of MyBP-C is involved in the pathogenesis of HCM, then controlling its expression or favouring its down regulation can be

therapeutically important. ACE inhibitors as well as ARBS are common drug of prescription in HCM and have the ability to induce down regulation of MyBP-C expression [52].

We have previously seen how the C0C1 fragment of MyBP-C can be used as a potential biomarker for diagnosis in acute myocardial infarction. C0C1 fragment has pathogenic effect on cardiac function [53] and can lead to cardiac dysfunction and HF.[38] C0C1 fragments decrease the viability of cells, decease calcium sensitivity, shorten the sarcomere length and enhance poor interaction with actomyosin, thus significantly affecting the systolic and diastolic function.[53] Moreover, once released into the circulation, it can provoke autoimmune response that can lead to autoimmune myocarditis and ultimately DCM.[43] Thus, a therapeutic strategy-targeting fragment C0C1 may be protective from all the mentioned above adverse cardiac consequences of AMI.

CONCLUSION

MyBP-C has become a protein of interest clinically. It is important to understand the structure and functions of MyBP-C, which will enable us to better elucidate its involvement in pathogenesis of certain cardiac conditions such as cardiomyopathies and heart failure. Studying its release, kinetics and clearance from the circulation and by comparing to other conventional biomarkers, it has been shown that MyBP-C C0C1 fragments can be used as a novel biomarker for AMI. Moreover, potential therapeutic strategies targeting MyBP-C can be designed.

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