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Cardiac myosin binding protein-C: a potential early-stage, cardiac-specific biomarker of ischemia-reperfusion injury

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Cardiac myosin binding protein-C (cMyBP-C) regulates sarcomeric structure, as well as myocardial contractility, and its phosphorylation is known to confer cardioprotection. We recently showed that cMyBP-C is an easily releasable and soluble myofilament; dephosphorylation of cMyBP-C results in its degradation and release into the blood post-myocardial infarction (MI); and plasma cMyBP-C levels are significantly increased in animal models and patients with MI. Strikingly, the level of plasma cMyBP-C is significantly (twofold) higher than the gold-standard plasma cardiac troponin-I (cTnI). This editorial provides an opportunity to discuss cMyBP-C as a potential new biomarker signaling ischemia-reperfusion injury. It is, however, clear that defining cMyBP-C as a *bona fide* selective and cardiac-specific biomarker of ischemia-reperfusion injury, as well as validating precise plasma cMyBP-C levels, will require further studies using systematic and advanced proteomics methods.

Acute coronary syndrome (ACS) encompasses all conditions that are caused by a sudden inadequate perfusion of the heart. This can occur through a decrease of blood flow or increased demand to the heart. ACS includes ST-segment elevation MI (STEMI), non-STEMI (NSTEMI) and unstable angina [1]. Symptoms can vary from classic crushing chest pain that radiates down the left arm to non-descript jaw or back sensations. Every 25 s, approximately one American will experience ACS with an estimated 34% chance of dying within 1 year subsequent to the ACS event [1]. An ECG provides immediate diagnosis of STEMI, activating a fast 90-min treatment pathway from first medical contact to opening the blocked coronary artery (i.e., 'door to balloon time') [2]. However, ST-elevation could be due to other causes such as pericarditis, early repolarization and ventricular hypertrophy. More importantly, life-threatening NSTEMI/unstable angina can still be missed due to a nondiagnostic ECG. Furthermore, the incidence of NSTEMI has increased (from 126 to 132 per 100,000), while the incidence of STEMI has decreased (from 121 to 77 per 100,000) between 1997 and 2005 [3]. Moreover, NSTEMI shows greater 1-year mortality (18.7–27.6%) than STEMI (8.3–15.4%) [3]. When a patient presents with ACS, but without ST-

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segment elevation, a clinician has to decide on either an early invasive or conservative approach based on assessment of the patient's risk [4,5]. Currently, this decision process can be difficult. The Timing of Intervention in Patients with Acute Coronary Syndromes (TIMACS) trial has shown that early invasive therapy decreases the possibility of death, MI and stroke in higher risk NSTEMI/unstable angina patients compared with standard care, with longer time to invasive therapy [6]. The American Heart Association (AHA) guidelines [4,5], the Global Registry of Acute Coronary Events (GRACE) score [4] and the Thrombolysis in Myocardial Infarction (TIMI) risk score [4] all require a positive circulatory biomarker as an indicator of high risk of heart attack. Consequently, biomarkers play a crucial role in risk-stratifying a NSTEMI ACS patient for proper care [4,5]. In this time-critical context, cMyBP-C has the potential to outperform cardiac troponins at identifying patients needing early invasive therapy and can be used to diagnose recurrent MI, which is not possible with troponins as they cannot diagnose delayed clearance.

Limitations of cardiac troponins

Professional medical organizations worldwide have agreed upon a universal definition of MI [7]. This universal MI definition prescribed cTnI or cardiac troponin-T (cTnT) as the preferred biomarkers to diagnose MI at values >99th percentile of normal [7]. However, elevation of cardiac troponins can be delayed by up to 8–12 h [4]. Highly sensitive cardiac troponin assays used for earlier detection only have sensitivities <85% for chest pain onset within 3 h of test; therefore, many MI cases will be missed within the first 3 h of chest pain onset at patient presentation [8,9]. Conversely, a wide range of positive predictive values (42–83%) will also produce many false positives [8,9]. Furthermore, lowering the threshold below 99% to increase sensitivity will further decrease specificity, resulting in even lower positive predictive values [8,9]. Thus, a need exists for a better biomarker to identify higher risk NSTEMI patients who can benefit from early invasive intervention [6], while avoiding performing potentially harmful procedures on non-ACS patients.

cMyBP-C: a new diagnostic tool for MI

The newest potential cardiac-specific marker for the detection of MI is cMyBP-C [10]. It is a thick filament assembly protein in the sarcomere that interacts with titin, myosin and actin to regulate the structure and function of the heart [11–15]. Specifically, cMyBP-C is responsible for the cross-linkages of myosin in the A-band region of the sarcomere. This is accomplished when cMyBP-C is phosphorylated by a number of different kinases, such as PKA, PKC, PKD, CaMKII and RSK [15]. cMyBP-C phosphorylation is necessary for normal cardiac function [11,16]. Moreover, phosphorylated cMyBP-C protects the heart from myocardial injury [13]. Recently, we showed that the plasma level of cMyBP-C is significantly elevated in rats 3-days post-MI and in human patients with MI compared with healthy controls [10]. We also showed that cMyBP-C is an easily releasable myofilament protein from cardiac sarcomeres, and sensitive to proteolysis post- MI in a phosphorylation-dependent manner such that cleavage of its N-terminal fragments can be detected in plasma [10]. Strikingly, the level of plasma cMyBP-C was twofold higher than cTnI in human patients with MI, suggesting its potential as a valid biomarker for MI. More importantly, with cMyBP-C levels twofold higher than cTnI, there is a greater chance of achieving >99th percentile separation from normal at an earlier time point, thus increasing both the sensitivity and specificity necessary to detect NSTEMI. N-terminal regions of cMyBP-C are very sensitive to proteolysis during the early stages of ischemia, resulting in a corresponding early release of N-terminal fragments. In fact, our pilot studies indicated that cMyBP-C fragments can be detected in plasma within 30 min of ischemia in rats. These results provide further early evidence that cMyBP-C is a promising biomarker for MI. However, while cMyBP-C does have the potential to play a new role as an early-stage, cardiac-specific

biomarker, the time of release, half-life, peak concentration, association with severity of MI and post-translational modifications in the circulatory system still need to be determined.

I have been studying the structure and function of cMyBP-C since 1995, including the link between the *MYBPC3* gene as a factor in the etiology of hypertrophic cardiomyopathy and the association between cMyBP-C phosphorylation and contractile function. In the last several years, I have taken this research in a new direction by exploring the potential of cMyBP-C as a potential biomarker for detecting early MI. Strong arguments support pursuing this research. First, cMyBP-C is highly soluble and very sensitive to proteolysis and, therefore, easily releasable from the sarcomere [10]. Based on these attributes, it promises to be a robust and early indicator of MI, compared with thin filament proteins such as cTnI and cTnT. These characteristics have been elucidated in our most recent manuscript [10]. Second, the N-terminal region of cMyBP-C is functionally critical to its roles in regulating sarcomeric structure and myocardial contractility. cMyBP-C provides longitudinal rigidity of the lattice and stiffness of the sarcomere. The arrangement of cMyBP-C in the sarcomere is different from in other thin and thick filaments [11,16]. Specifically, myosin, actin, cTnI and cTnT are arranged in the vertical axis in the sarcomere, whereas cMyBP-C runs through horizontally connecting all of the thin and thick filaments. This horizontal orientation may provide unique accessibility to proteases, which is currently under investigation [17]. Third, the N-terminal C0 domain of cMyBP-C is a unique cardiac isoform that is exclusively present in cardiac tissue. This is important in the context of biomarker discovery because the N-terminal fragments appear early in the blood post-MI [10]. Finally, recent studies from my laboratory have demonstrated that dephosphorylation of cMyBP-C accelerates its degradation and cleavage of the N-terminal region, leading to early release into the circulatory system. Based on these lines of evidence, I hypothesized that the plasma cMyBP-C level, as detected as both a full-length peptide and fragment, can be a clinical champion and provide a more robust and error-free indication of MI.

Conclusion & future perspective

My group's studies were dependent on the traditional sandwich ELISA to quantify the amount of plasma cMyBP-C; therefore, a sensitive tandem-mass spectrometry technique known as selective reaction monitoring (SRM) will be required to determine the precise amount of cMyBP-C in the plasma samples. The efficacy of cardiac-specific markers of myocardial ischemia or necrosis in heart failure remains unclear, but literature and technology in this area are compelling. Recent advances in the field of clinical proteomics and the application of innovative technologies, such as functional genomics and proteomics, have greatly accelerated the discovery, verification, validation and application of novel biomarkers, particularly cardiac-specific biomarkers, as the best way to titrate therapeutic intervention [18]. cMyBP-C is a large protein, and cleaved in many regions during proteolysis. Thus, to perform a systematic determination and validation of cMyBP-C as an early biomarker for MI, an ultrasensitive proteomic assay that can capture different regions of full-length cMyBP-C in one reaction is proposed. Specifically, the use of mass spectrometry-based proteomics has many advantages over antibody-based ELISA approaches, such as quantification accuracy, site specificity, sensitivity and a broad coverage of proteins [19,20]. Also, while traditional mass spectrometry attempts to detect all proteins in a biological sample in a shotgun fashion, SRM, as noted above, is highly targeted, allowing scientists to quantitate specific peptides of interest [18,21]. In fact, SRM is now routinely applied for the verification of candidate biomarkers from discovery experiments, ensuring that only highly qualified candidates move into clinical validation [22,23]. Moreover, the SRM approach will allow for greater sensitivity, specificity, quantitation and speed of evaluation of cMyBP-C as a biomarker candidate [22–25]. Future studies will demonstrate that the plasma level of cMyBP-C is an index of cardio-

pathophysiological change, providing a tool for clinicians to more accurately diagnose and monitor ischemic injury, and for researchers to conduct biomarker discovery with novel proteomics approaches. These findings are anticipated to open up a new avenue of diagnostic and therapeutic investigation.

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Biography

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