Review

Troponin I, cardiac diastolic dysfunction and restrictive cardiomyopathy[1]

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ABSTRACT

Cardiomyopathies are diseases of heart muscle that are associated with cardiac dysfunction. Molecular genetic studies performed to date have demonstrated that the damage or mutations in several sarcomeric contractile protein genes are associated with the development of the diseases. In this review, cardiac troponin I, one of the sarcomeric thin filament protein, will be discussed regarding its role in cardiac function, its deficiency-related diastolic dysfunction, and the mutation of this protein-mediated restrictive cardiomyopathy.

INTRODUCTION

Heart failure is a major and escalating public health problem in many countries. Despite advances in various treatments, the number of patients with chronic heart failure is increasing[1]. Cardiomyopathies are diseases of heart muscle that are associated with cardiac dysfunction. Heart failure due to cardiomyopathy represents a major health problem. Therefore, elucidation of the fundamental mechanisms responsible for the development of cardiomyopathy is important.

Cardiomyopathies have traditionally been considered to represent diseases that primarily affect cardiac muscle, although in many cases cardiac dysfunction arises due to a secondary effect on the heart from an initial insult outside of cardiac muscle *per se*. As such, the pathways that drive the onset and progression of the disease often reflect the combined effects of genetic susceptibility, environmental stimuli, and hormonal and physiological cues from other organ systems[2]. On the ground of their morphology and pathophysiology, cardiomyopathy may be classified into three major types: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and restrictive cardiomyopathy (RCM). In 1996, the World Health Organization (WHO) International Society and Federation of Cardiology Task Force added 2 more classes: arrhythmogenic right ventricular cardiomyopathy and unclassified cardiomyopathies[3]. The description of DCM and HCM cardiomyopathies is based primarily on morphological criteria, as their names imply. However, RCM is a primary abnormality of diastolic dysfunction caused by derangement in the dynamics of ventricular filling, resulting in an increase in ventricular end-diastolic pressures and dilated atria. Over the last decade, the importance of gene defects in the etiology of primary cardiomyopathies has been recognized. Autosomal dominant, autosomal recessive, X-linked, and maternal patterns of in-

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heritance have been observed. Families with inherited cardiomyopathies have provided a unique resource for studies of the genetic basis of these disorders. Molecular genetic studies performed to date have focused largely on monogenic inherited cardiomyopathies, ie, caused by mutations in a single gene. Some cardiomyopathies, such as familiar hypertrophic cardiomyopathy, are now characterized as "sarcomeric diseases" on the basis of evidence that mutations in sarcomeric proteins are causal. The contractile sarcomeric proteins consist of a highly ordered arrangement of myosin thick filaments, actin thin filaments, and associated proteins, such as the troponin-tropomyosin complex. Several different disease genes have been identified for cardiomyopathies[4]. In this review, we will discuss the role of one of the sarcomeric proteins, troponin I in cardiac function, its deficiency-related diastolic dysfunction, and restrictive cardiomyopathies associated with cardiac troponin I mutation.

STRUCTURE AND FUNCTION OF TROPONIN

Sarcomere contractile proteins Sarcomeres are the contractile units of striated muscles. Each sarcomere unit is 2.2 mm in length and is attached to its neighboring sarcomeres through the Z discs. Sarcomeres are composed of more than 15 different proteins that are arranged into thick, thin, and intermediary filaments. Thick filaments are formed through the assembly of several hundred myosin heavy chain (MHC) molecules that are attached together through tail-to-tail inter-binding. Other components of the thick filaments are myosin binding protein C (MBP-C), which binds to myosin and titin, and myosin light chain-1 (MLC-1), and myosin light chain-2 (MLC-2) proteins, which are attached to the head of the MHC protein. Thin filaments are composed of actin, the troponin complex (troponin T, TnT; troponin C, TnC; and troponin I, TnI), and a-tropomyosin in a 7:1:1 molar ratio. Another important sarcomeric protein is titin. Titin spans the entire length of the sarcomere and provides elasticity to the contractile units.

Troponin complex and muscle contraction Troponin regulates skeletal and cardiac muscle contraction. Troponin has three subunits: TnT, the tropomyosin-binding subunit; TnC, the calcium binding subunit, and TnI, the subunit which inhibits cross-bridging of actin and myosin at sub-activating concentrations of calcium. During the process of contraction of a myocardial cell, calcium is released from the sarcoplasmic reticulum and becomes available to the myofilaments. Calcium binds to a regulatory calcium site on the troponin complex, the TnC subunit. The presence of calcium at this site is known to result in a change in affinity of TnC for TnI, and there are resulting interactions with other thin filament proteins such that cross-bridging of myosin heads to actin becomes more favorable. When a cross-bridge is bound, the myosin head may complete a cycle that results in sliding of the thick and thin filaments in opposite directions and muscle shortening. The detachment and stroke of the myosin head requires hydrolysis of ATP.

Inhibitory subunit, TnI, and muscle relaxation Cardiac TnI is a basic globular protein containing approximately 210 amino acids (24 kDa). TnI interacts with both tropomyosin and actin[5,6] and is responsible for the inhibition of the Mg^{2+}-ATPase activity of actomyosin[7-10]. Inhibition of the actomyocin ATPase activity is neutralized when calcium-saturated TnC forms a complex with TnI[10-12]. Syska *et al* first demonstrated that three TnI fragments, CN4 (residues 96-117), CN5 (residues 1-21), and CF2 (residues 1-47), were all capable of binding to a TnC-Sepharose affinity column but only the CN4 fragment (residues 96-117) was capable of interacting with acto-tropomyosin and inhibiting the acto-S1-TM ATPase activity[13]. TnI CN4 fragment (residues 96-117) has gained attention for extensive investigation and it demonstrated that TnI residues 104-115 (Ip) comprised the minimum sequence necessary for the inhibition of actomyosin ATPase activity[14,15]. It has been concluded that the Ca^{2+}-dependent switch between muscle relaxation and contraction involves a switching event of the TnI inhibitory region (residues 104-115) between actin-TM and TnC, respectively[16]. Gel electrophoresis and high performance liquid chromatography (HPLC) studies have demonstrated that the sequences of the N- and C-terminal regions of TnI interact in an anti-parallel fashion with the corresponding N- and C-domain of TnC. The N- and C-terminal domains of TnI which flank the TnI inhibitory region (residues 104 to 115) play a vital role in modulating the Ca^{2+}-sensitive release of the TnI inhibitory region by TnC within the muscle filament[17].

Phosphorylation of serine residues in the N-terminal extension unique to cardiac TnI (cTnI) is known to occur through protein kinase A (PKA). These serine residues play an important physiological role in cardiac contraction. When phosphorylated, cTnI induces co-

operative interactions in the thin filament that reduce the calcium affinity of TnC, an effect that accelerates relaxation when the heart is influenced by β-adrenergic agonists. Protein kinase C (PKC) has been shown to phosphorylate cTnI at Ser-43/Ser-45, Ser-78, Thr-144, and other undetermined sites[18] and phosphorylation of these multiple sites is associated with an inhibition of calcium-stimulated actomyosin MgATPase[19,20]. The effect of phosphorylation of the specific sites is unknown.

TROPONIN I DEGRADATION AND CARDIAC STUNNING

Myocardial ischemia/reperfusion presents a spectrum of injury, from reversible damage with mild ischemia, to irreversible cellular necrosis with severe ischemia. Several studies have demonstrated selective TnI degradation under ischemia/reperfusion injury[21-23]. TnI has been observed being proteolytically cleaved during ischemia and ischemia/reperfusion[22,24]. McDonogh *et al* have demonstrated that with moderate ischemia/reperfusion, cTnI is degraded at its C-terminal end by removal of 17 amino acids generating $cTnI_{1-193}$[25]. With longer durations of ischemia and ischemia/reperfusion, there is also an N-terminal truncation generating two other degraded forms of the protein ($cTnI_{63-193}$ and $cTnI_{73-193}$).

Stunning is a clinical syndrome, a phenomenon in which patients experience reversible contractile dysfunction of the heart following a transient episode of ischemia and subsequent reestablishment of blood flow. It is considered an acute injury, although its severity varies with the length of the ischemic episode. As such, instances of stunning may appear morphologically and functionally similar, yet need not arise necessarily from the same molecular mechanisms[26]. Kusuoka and Marban first suggested that stunning was associated with reversible breakdown and replacement of damaged myofilament proteins[27].

The importance of cTnI proteolysis over other protein modifications is suggested by its crucial role in the control of calcium-dependent muscle contraction. Recently, the transgenic mice were produced that express $cTnI_{1-193}$, the cTnI degradation product found in stunned rat cardiac myocytes[28]. These mice exhibited ventricular dilation and decreased myofilament contractility, demonstrating that cTnI proteolysis alone was sufficient to recapitulate the cellular phenotype of stunning[28]. McDonough *et al* analyzed biopsy samples from 37 bypass patients and found that TnI was specifically and selectively modified in the myocardium of the bypass patients[25]. However, *in vivo* swine and canine models of stunning have failed to demonstrate stunning-dependent cTnI degradation[29,30]. Moreover, the mechanism by which cTnI degradation causes contractile dysfunction and cardiac stunning is not clear.

DIASTOLIC DYSFUNCTION DUE TO CARDIAC TROPONIN I DEFICIENCY

TnI isoform switching during heart development cTnI and slow skeletal TnI (ssTnI) are two main TnI genes expressed in the mammalian heart under the control of a developmentally regulated program[31-33]. The ssTnI is expressed during embryonic and early postnatal life and is then replaced entirely by the cTnI two weeks after birth[34,35]. Despite overall similarity, there are a number of significant structural differences between ssTnI and cTnI. Perhaps most importantly, cTnI has a 27-33 amino acid N-terminal extension which is absent in ssTnI and which contains two cAMP-dependent protein kinase (PKA) phosphorylation sites. These sites have been shown to be phosphorylated in response to β-adrenergic receptor stimulation and have been postulated to play important roles in regulating TnI-TnC interactions, the co-operative binding of troponin to actomyosin, and the affinity of the regulatory sites of TnC for Ca^{2+}[36-39]. The role of cTnI phosphorylation by PKA has been further confirmed very recently in ssTnI transgenic animals[40-42].

Human cTnI and ssTnI genes have been cloned and characterized[43,44]. The similarity between human and mouse TnI genes is very high, suggesting that this gene is highly conserved in various species. It also suggests that the mouse is a good model to investigate the cardiac function of TnI in the heart.

Troponin I deficiency and cardiac dysfunction Alterations in gene expression are a feature of cardiac dysfunction. Experimental cardiac hypertrophy and heart failure in the rat are associated with the re-expression of many genes normally observed during fetal cardiac development[45,46]. However, there is no evidence that ssTnI is re-expressed in experimental mouse, rat hearts with cardiac hypertrophy[47,48] or in human hearts in end-stage heart failure[35]. It is clinically important to understand the TnI function and TnI gene regulation, because TnI may become depleted in ischemic[24], infarcted[21,49], and possibly failing myocardium[50]. Recently, a research group has examined cardiac au-

topsy samples from patients and reported that the concentration of cTnI in left ventricular myocardium is 7.7-fold lower in older men compared to that of young men (<age 35 a)[51].

A cTnI gene knockout mouse model was created by using gene targeting techniques[52]. It provides us with a unique loss-of-function disorder model to study the role of cTnI in contraction and relaxation. The result of myocardial TnI deficiency is remarkably consistent cardiomyopathy with diastolic dysfunction resulting in sudden death in cTnI gene knockout mice on d 17-18 after birth. A significant characteristic in cTnI null cardiac myocytes is a shortening of sarcomere length even in relaxing solution. This shortening of sarcomere under relaxed conditions indicates the presence of calcium-independent forces in the direction of normal active muscle contraction. The elevated force could prevent a complete relaxation of cardiac myofilaments. That means a decrease in cardiac compliance and an increase in cardiac stiffness resulting in a damaged cardiac dilation. Those cTnI gene knockout mice died in their early life of heart failure due to diastolic dysfunction[52]. Disturbance of energy metabolism in cardiac myocytes from cTnI null mice was also observed[53].

CARDIAC TROPONIN I MUTATION AND RESTRICTIVE CARDIOMYOPATHY

The contractile sarcomeric proteins consist of a highly-ordered arrangement of myosin thick filaments, actin thin filaments, and associated proteins, such as the troponin-tropomyosin complex. The repeating sarcomere units are arranged in series, resulting in striated myofibrils. Several different disease genes have been identified for hypertrophic cardiomyopathy, one of the most frequently occurring inherited cardiac disorders[54] and the first to be elucidated at a molecular genetic level. The genes encode proteins which are components of either the thick filament (β-myosin heavy chain, essential myosin light chain, regulatory myosin light chain and myosin binding protein-C) or the thin filament (cardiac troponin T and I, α-tropomyosin) of striated muscle[55,56]. The disease is clinically heterogeneous, with some mutant alleles resulting in a poor clinical prognosis and others being relatively benign[57]. The fact that so many of the HCM mutations are linked to genes encoding sarcomeric proteins suggests that the pathogenesis of the disease likely involves a common mechanism of contractile dysfunction.

Cardiomyopathies are defined as diseases of the myocardium associated with cardiac dysfunction. Linkage studies have demonstrated that HCM was closely related to the mutation and damage of myofibril proteins such as myosin heavy chain, troponin T, tropomyosin, and troponin I[58-60]. Actin mutation has been reported to be associated with DCM[61].

Among various types of cardiomyopathies, RCM manifests itself as the restrictive filling and reduced diastolic volume of either or both ventricles with normal or near-normal systolic function and wall thickness. In RCM patients, the heart is abnormally stiffened which may be idiopathic or associated with other disease (eg, amyloidosis; endomyocardial disease with or without hypereosinophilia)[3]. A retrospective study of 106 cats was carried out in the Feline Center of the University of Bristol, UK. It has revealed that HCM was the most common form seen (57.5 %) followed by RCM (20.7 %), DCM (10.4 %), and unclassified cardiomyopathy (10.4 %)[62].

Some case reports have described familial restrictive cardiomyopathy in human[63,64]. Both autosomal dominate and autosomal recessive patterns of inherited RCM have been described[65,66]. Very recently, the clinical features of RCM were described as a marked elevation of left ventricular end-diastolic pressure and profoundly abnormal myocardial relaxation. The patient died suddenly at the age of 13 months[67]. A French medical group also reported a case of restrictive cardiomyopathy which may be related to myofibrillar myopathy[68]. A common feature of RCM for those reported cases is a poor outcome with a sudden death. The prognosis in RCM varies according to the underlying cause, but the majority of individuals experience progressive deterioration due to congestive cardiac failure with a high incidence of premature mortality. Clinically, the endomyocardial biopsy observation can be used for differential diagnosis of RCM from constrictive pericarditis. However, the etiology of RCM was still not clear. Some studies have revealed that RCM is associated with myofibrillar proteins such as desmin[65,69]. Symptomatic improvement in RCM may be achieved with diuretic or vasodilator therapy[4].

Very recently, a linkage study on a RCM patient's family and 32 of his surviving relatives has demonstrated that idiopathic RCM was part of the clinical expression of cardiac TnI mutation[70]. Subsequent mutation analysis revealed a novel missense mutation, which cosegregated with the disease in the family (lod score: 4.8). To

determine if idiopathic RCM is part of the clinical expression of cTnI gene mutations, genetic investigations of the gene were performed in an additional nine unrelated RCM patients with restrictive filling patterns, biatrial dilation, normal systolic function, and normal wall thickness. cTnI gene mutations were identified in six of these nine RCM patients. Two of the mutations identified in young individuals were *de novo* mutations. All mutations appeared in conserved and functionally important domains of the gene. The identification of cTnI gene mutations in idiopathic RCM patients indicates that this phenotype is part of the spectrum of hereditary sarcomeric contractile protein disease[70].

TnI mutation has also been linked to hypertrophic cardiomyopathy in human patients[71,72]. The main discovery is the mutation R145G on human cTnI gene[60,73]. However, other mutations, such as G203S, on this gene have also been reported that are related to human hypertrophic cardiomyopathy[60]. It is not clear thus far what the signal pathway is between the mutations on the cTnI gene and the development of hypertrophic cardiomyopathy.

DISCUSSION AND SUMMARY

Among four types of cardiomyopathies, RCM cases are not so common as that of HCM, but the prognosis is poor. RCM is initially manifested by reduced ventricular compliance or increased stiffness. cTnI occupies a central position at the crossroads of the signaling pathways between calcium binding to cTnC and crossbridge binding to actin. This determines the critical role of cTnI in cardiac contraction and relaxation. As an inhibitory element, cTnI prevents the interaction between myosin heads and actins, ie, the formation of crossbridges, at sub-activating concentrations of calcium. This ensures the muscle relaxation, ie, cardiac relaxation or cardiac dilation. It is not surprising to observe that cTnI degradation, cTnI mutation or truncation, and cTnI deficiency could affect myofilament function. In fact, both experimental studies and human patients investigations have shown a tight correlation between cTnI alterations (degradation, mutation and deficiency) and cardiac dysfunction, in particular, diastolic dysfunction, a damaged cardiac dilation and restrictive cardiomyopathies. The relationship between cTnI gene mutations and idiomathic restrictive cardiomyopathies obtained from a human patient investigation[70] is very important, since it provides us a genetic clue for the cause of the disease. Since the cTnI mutations that cause RCM have been identified only recently, the continued characterization of patient mutations and their phenotypes is necessary to establish clinically meaningful correlation. In addition, the development of animal models linking the genetic mutation to functional defects are critical for understanding how the point mutation alters the protein structure resulting in the alteration of myofilament structure and the alteration of cardiac function.

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