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Review

Sarcomeric protein isoform transitions in cardiac muscle: A journey to heart failure

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ABSTRACT

Sarcomeric protein isoforms are mainly governed by alternative promoter-driven expression, distinct gene expression, gene mutation and alternative mRNA splicing. The transitions of sarcomeric proteins have been implicated to play a role in the onset and development of human heart failure. In this mini-review, we summarized isoform transitions of several most widely examined sarcomeric proteins including myosin, actin, troponin, tropomyosin, titin and myosin binding protein-C, and the consequence of these abnormal isoform transitions. Even though the isoform transitions of sarcomeric proteins have been described in individual sarcomeric protein reviews, no concise summary of these results has been presented previously. This review is intended to fill this gap and discuss possible future perspectives.

1. Introduction

Heart failure (HF) has been a major public health focus with an escalating health threat [1]. A failing heart is characterized by unfavorable alterations in myocardial contraction (systolic dysfunction) and/or impaired ability of ventricular filling (diastolic dysfunction). Heart failure is a complex clinical syndrome attributed to a number of factors such as changes in cardiac structure, for example, dilation and hypertrophy, abnormal energy metabolism, intracellular calcium handling defects, and sarcomeric dysfunction. Recent studies revealed that alterations in sarcomeric function play a cardinal role in the development of heart failure [2,3].

Sarcomeric function is determined by post-translational modifications and by the levels of multiple isoforms of sarcomeric proteins. The most abundant sarcomeric proteins are contractile proteins/myofilament proteins (myosin and actin), regulatory proteins (troponins and tropomyosin) and cytoskeletal proteins (myosin binding protein C and titin). All of these proteins are located in the sarcomere, and take up approximately 80% of the myofibril mass. In one sarcomere unit, N-terminus of titin anchors to Z-disk and C-terminus of it attaches to M-line, which spans half sarcomere from the Z-disk to center of the sarcomere. Titin is attached to both actin and myosin and is anchored to the Z-disk (Z-line). Myosin is in the center of the sarcomere. Actin is cross-linked in the Z-lines and overlaps with myosin (Fig. 1A) [4,5]. Fig. 1B is a diagram of the thin filament and thick filament in cardiac muscle indicating the four major components: myosin, actin, tropomyosin (Tm) and troponin complex (TnT, TnC and TnI). During muscle contraction, binding of calcium ions to TnC triggers a conformational rearrangement in the troponin complex, and then the movement of tropomyosin exposes a space for myosin binding on actin, which allows cross-bridge formation driven by the energy provided from ATP hydrolysis [6,7]. The impairment of these proteins will result in the dysfunction of muscle contraction, which is associated with transition to heart failure. Recent evidence depicted that aberrant expression levels and transition of sarcomeric protein isoforms are associated with sarcomeric dysfunction. The current review describes isoform alterations of the major sarcomeric proteins and the resulting functional consequences in cardiac muscle.

2. Myofilament proteins

Myofilament proteins make up the bulk of the sarcomere. Actin and myosin are the major contractile proteins of the thin and thick filaments, respectively. They are responsible for force generation during muscle contraction.

2.1. Myosin

The myosin motor molecule generates force and motion by coupling its ATPase activity to its cyclic interaction with actin. Myosin consists of two myosin heavy chains (MHCs) and two essential and two regulatory myosin light chains (MLCs) [8]. Mammalian cardiac myosin heavy

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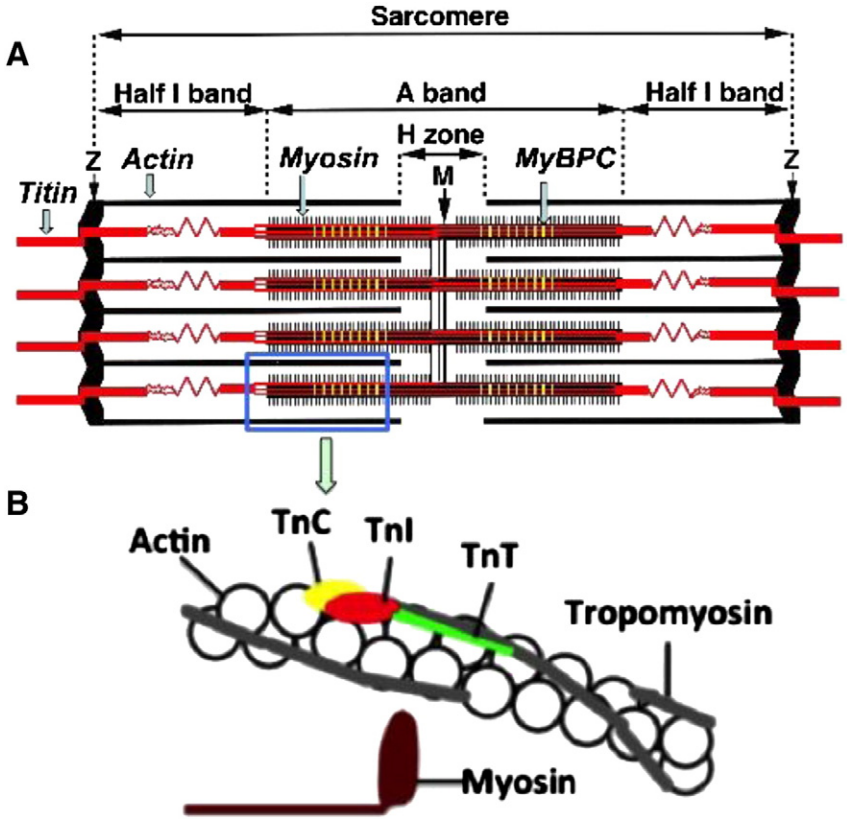

Fig. 1. The location and arrangement of the most abundant proteins in the cardiac sarcomere, the basic contractile unit. A, Titin anchors each half sarcomere from the Z-line to the M-line; the N-terminal I-band region of titin is bound to actin that is located to Z-line at its N-terminus and attached to myosin at its C-terminus; the C-terminal A-band segment of titin is attached to the thick filament via multiple binding sites for myosin and myosin binding protein C (C-protein); B, The thin filament is composed of actin, tropomyosin, and the troponin complex (Tn-T, Tn-C, and Tn-I). The thick filament is composed of myosin and C protein; during muscle contraction, calcium binding to Tn-C induces a conformational rearrangement in Tn-C, causing the Tn-C:Tn-I association to strengthen while at the same time weakening the Tn-I:Tn-T association; movement of tropomyosin exposes a myosin binding site on actin allowing cross-bridge formation driven by the energy from ATP hydrolysis.

chains have two isoforms: α-isoform and β-isoform encoded by two distinct genes [9]. In rodent hearts, the α-MHC is the major isoform of the ventricular myocardium (~90%) [10], while the β-isoform is predominant in human ventricular myocardium (~95%) [11]. The mammalian α-isoform is associated with higher actomyosin ATPase activity than the β-isoform, therefore, hearts expressing α-isoform possess more rapid contractile velocity than hearts expressing β-isoform. The β-isoform imparts greater economy in force generation because the tension time integral for force per cross-bridge cycle is greater [12].

Cardiac MHC isoform expression is species-dependent, developmentally controlled, and sensitive to hormonal perturbations and cardiovascular stress [13,14]. The shift or transition between cardiac MHC isoforms might play an important role in cardiac function. The transition of cardiac MHC isoforms has been observed in hyperthyroid compared with euthyroid rats, which the transition is from the slow β-isoform to the fast α-isoform [15]. The transition from the slow β-isoform to the fast α-isoform has also been found in human failing myocardium [3,10], and thyroid depletion, aging, cardiomyopathy, and pressure overload have been shown to increase β-isoform [16]. Additionally, down-regulation of α-isoform and up-regulation of β-isoform occur during experimental induction of heart failure in mammalian myocardium that normally expresses predominantly α-isoform [17]. A regional shift of cardiac MHC isoforms has been observed in human and rat ventricular tissues, with the fast α-isoform exhibiting a higher expression in the subepicardial than in the subendocardial layer [18]. This regional transition of MHC isoforms is consistent with contraction duration and the shorter action potential in the sub-epicardium compared with sub-endocardium. Recent reports have demonstrated that a slight shift of isoforms will have a significant influence on cardiomyocyte contractility [19]. In contrast to human ventricular tissues, atria human tissues contain ~80% of α-isoform [20]. In atrial fibrillation, expression of β-isoform is increased almost two-fold [21], and in failing right and left ventricles, α-isoform was expressed in a significant lower level [20]. Overall, aberrant isoform switching between α-isoform and β-isoform may likely occur in diseased human atria and ventricular myocardium.

The isoform shift also occurs in MLCs within the heart. Particularly, isoform alterations have been reported for MLC-1 (essential MLC) both in atrial and ventricular myocardium. Essential MLC (MLC-1) has two isoforms: a ventricular form (VLC-1) and an atrial form (ALC-1) [22]. The entire heart muscle expresses ALC-1 during fetal and early life and is subsequently replaced by VLC-1. Patients with hypertrophic obstructive cardiomyopathy in ventricles express a higher ratio of ALC-1 to VLC-1 [23]. However, the isoform ratios between ALC-1 and VLC-1 display much larger individual differences in ischemic and idiopathic cardiomyopathy (IDCM) [22,23]. In addition, cardiac function can be benefited by the addition of the N-terminal region of MLC-1 via certain mechanisms that the N-terminus of MLC-1 peptide directly influences interaction of proteins and exerts an inotropic effect via cooperative mechanisms, which activate the entire thin filament [22]. Therefore, up-regulation of the N-terminal fragment of MLC-1 isoform might provide a therapeutic approach to strengthen cardiac performance.

2.2. Actin

Actin is essential for a very wide range of cell functions. Mammalian actin isoforms are highly conserved and ubiquitously found in

eukaryotic cells. There are six actin genes in the human genome that encode six different actin isoforms. Four isoforms, α-cardiac actin, α-skeletal actin, α-smooth actin, and γ-smooth actin, are present respectively in cardiac, skeletal, and smooth muscles, while the other two isoforms, named β-cyto actin and γ-cyto actin, are ubiquitously expressed in tissues and cells. These isoforms are extremely similar to each other, with exclusive small variations in the protein sequence [24]. The four muscle actins are restricted to tissues with high tonic activity such as striated heart muscle, skeletal muscle and smooth muscle [25]. The α-cardiac actin is the predominant isoform in adult hearts, accounting for approximately 80% of total actin in both human ventricular and atrial samples. The α-skeletal actin is found to be about 20% in adult myocardium [24]. The early expression of the α-smooth isoform in the fetal human heart and its replacement by α-skeletal actin suggests that these proteins may play distinct roles in myocyte growth and development. The subtle changes of these isoform ratios may be causing important differences in contractility. Cardiac contractility has been proved to be affected by the expression ratio of α-skeletal actin and α-cardiac actin [24]. Moreover, there is evidence to indicate that ectopic expression of γ-smooth actin and decreased α-cardiac actin expression reduce cardiac contractility [26]. Combined with other results, they may explain why large mammals such as humans normally have much higher levels of α-skeletal actin in their hearts than small mammals such as rats and mice [27]. In an animal model, α-cardiac actin deficiency leads to lethality at embryonic or perinatal stage with profound disarray of cardiac myofibrils [28]. α-skeletal actin deficient mice appear healthy at birth, however, all of these mice die with weak muscles by 9 days of age [29]. α-Smooth actin deficient mice appear normal and are viable but have functional impairment in blood pressure regulation and vascular contractility [30]. The transgenic animal models indicated that over-expression of γ-smooth actin only partially rescued mice lacking α-cardiac actin, suggesting that γ-smooth actin and α-cardiac actin each make divergent contributions to cardiomyocyte function [28]. Up-regulation of α-cardiac actin in α-skeletal actin knockouts fully rescued the lethality and muscle function defects, which suggests a potential treatment for heart failure associated with reduced α-skeletal actin [29]. For the more detailed discussion on actin isoforms the readers are referred to some recent reviews [25,31].

3. Regulatory proteins

Muscle contractility is controlled by sarcoplasmic Ca^{2+}, which acts on the receptor molecule troponin in thin filaments [7]. Ca^{2+} is bound to troponin C that in turn binds to troponin I, releasing it from its inhibitory site on actin. Second, the TnT binding to Tm and the overlap of Tm from adjacent thin filament regulatory units allow coupling and propagation of Tm motion along the thin filament. Finally, cross-bridge binding contributes to activation by stabilizing the Tm/Tn unit in an activated position. The extent of this coupling, or cooperative activation, varies with the composition of the regulatory protein isoforms [7].

3.1. Tropomyosin

Tropomyosin is formed as a homodimer or heterodimer of two α-helical chains arranged as a coiled coil. The composition of tropomyosin isoforms in actin filaments plays a crucial role in functional regulation of actin filaments in either muscle or non-muscle cells. This family is often divided into two groups as muscle tropomyosin isoforms and non-muscle tropomyosin isoforms [32]. Tropomyosin isoforms are involved in the regulation of interactions between actin and myosin in the muscle sarcomere and play a pivotal role in modulating the kinetics of activation of muscle contraction [33]. In humans, tropomyosin isoforms are encoded by four highly conserved genes (*TPM1*, *TPM2*, *TPM3* and *TPM4*). Each of them can produce multiple isoforms through the use of different promoters, alternative splicing, and different sites of poly (A) addition signals [34]. The principal tropomyosin isoforms found in human striated muscles are α-tropomyosin and κ-tropomyosin from *TPM1* gene, β-tropomyosin from *TPM2* gene, and γ-tropomyosin transcribed from *TPM3* gene. In adult human hearts, β to α-tropomyosin ratio is about 1:4.8, while in large animals such as beef, pig and sheep this ratio is present at approximately 1:4, and in small animals like rabbit, rat and dog only the α-isoform is expressed in the heart [35].

There are very few reports that the isoform transition of Tm is associated with human heart disease. To the best of our knowledge, one study has reported that failing heart ventricular muscle expresses exclusively α-tropomyosin isoform [36], and one recent study showed that increased expression of κ-tropomyosin isoform has been found in the hearts of patients with chronic dilated cardiomyopathy (DCM) [37,38]. However, in the rodent model, the α-tropomyosin isoform knockout mice showed an embryonic lethal phenotype, but in the heterozygotes, the knockout mice indicated normal phenotype as compared to control mice, suggesting that the levels of the protein are regulated at the translational level [39]. In a transgenic mouse model, the overexpression of β-tropomyosin isoform to 57.8 % of total heart tropomyosin demonstrates altered diastolic function with reduced rates of contraction and relaxation of isolated myocytes [40]. If the overexpression of β-tropomyosin isoform is over 75% of total heart tropomyosin, the mice die 10 to 14 days after birth and show severe cardiac abnormalities and thrombus formation [41]. In the mouse model overexpressing γ-tropomyosin isoform, the mice show increased heart rate and reduced Ca^{2+} sensitivity [42]. These data imply that tropomyosin isoform transition or composition alteration potentially affects cardiac function in humans.

3.2. Troponins

Troponin complex acts as a calcium-sensitive regulator of striated muscle contraction and consists of three regulatory proteins: the calcium binding protein troponin C (TnC), the inhibitory protein troponin I (TnI), and the tropomyosin-binding protein troponin T (TnT) [43].

TnC is encoded by at least two genes (*TNNC1* and *TNNC2*) found in the genomes of higher animals. *TNNC2* encodes the fast skeletal isoform (fsTnC), and *TNNC1* encodes a single TnC isoform that is expressed in slow muscle and in heart muscle. It is referred to as ssTnC in slow muscle and as cTnC in the heart [44]. In the heart muscle of higher vertebrates, only ssTnC/cTnC isoform has been expressed in both developing and adult hearts. So far, there have been no reports of troponin C isoform transitions during myocardial development or pathological adaptations [45].

TnI has three isoforms (ssTnI isoform in the slow skeletal muscle, fsTnI isoform in the fast skeletal muscle and cTnI isoform in the cardiac muscle) encoded by *TNNI1*, *TNNI2* and *TNNI3* respectively [46]. TnI is developmentally regulated in rat heart with co-expression of the ssTnI and cTnI in fetal heart. With development, the ratio of the ssTnI to cTnI is decreased, and the ssTnI is non-detectable in adult heart [46]. The developmental change of TnI isoforms in the human heart is similar to that in the rat heart. Each individual gene coded for the three TnI isoforms has no mRNA alternative splicing. The adult heart only expresses cTnI and doesn't undergo isoform switching to skeletal forms even under pathological conditions such as ischemic heart failure, dilated cardiomyopathy, and end-stage heart failure [47]. However, over-expression of slow TnI in cardiac muscle of adult transgenic mice impaired cardiomyocyte relaxation and diastolic cardiac function due to increased Ca^{2+} sensitivity [48].

TnT also has three muscle type-specific isoforms: the slow skeletal muscle TnT (ssTnT), the fast skeletal muscle TnT (fsTnT) and the cardiac muscle TnT (cTnT). These three isoforms are encoded by three genes: *TNNT1*, *TNNT3* and *TNNT2* separately [49]. Each TnT gene undergoes alternative splicing and generates multiple isoforms [45,49]. In human heart, there are at least four isoforms of cTnT (cTnT1, cTnT2, cTnT3 and cTnT4). The four isoforms of human cTnT are expressed in a

developmentally regulated manner [50]. Both cTnT1 and cTnT2 isoforms are expressed in the fetal heart, with a very low expression level of cTnT2. During the perinatal heart development, the expression level of cTnT1 decreases, and the expression of cTnT3 increases and appears as the only isoform in the normal adult heart. The cTnT4 isoform is also expressed in the fetal heart, but not in the adult heart; however, it is re-expressed in the failing adult heart [50]. Abnormal cTnT isoform expression has been linked to heart disease. The exon4 skipped isoform was increased in failing human heart [50], diabetic rat heart [51], and familial hypertrophic cardiomyopathy human hearts [52]. A shift in cardiac TnT splicing toward the fetal pattern appears in a rabbit model with mild cardiac hypertrophy [53]. In a guinea pig model with induced ventricular hypertrophy, four distinct TnT isoforms underwent changes in the relative proportions of the different isoforms during hypertrophy [54]. The skipping of conserved exon8 has been found in turkeys with inherited dilated cardiomyopathy and heart failure [55]. Aberrant skipping of exon7 appeared in cTnT of dog, pig and cat, indicating the high risk of dilated cardiomyopathy [56]. Overexpression of exon7-excluded cTnT isoform in the transgenic mouse heart leads to impaired systolic function [57]. Re-expression of the embryonic exon5 in adult heart was detected in dilated cardiomyopathy dogs [56]. The heterogeneity of TnT isoforms or co-presence of different TnT isoforms reduces cardiac performance by desynchronizing the calcium activation of thin filaments [58]. Previous studies have suggested that cardiac muscle contractile functions in adult mice were significantly impaired by co-existence of the endogenous cardiac TnT and overexpressed non-mutant fast TnT [59]. Further studies demonstrated that overexpression of one or more functionally distinct cardiac TnT isoforms in mice developed lower left ventricular pressure, decreased stroke volume, and produced slower contractile and relaxation velocities when compared to wild-type controls. These studies further imply that co-expression of functionally distinct cardiac TnT isoforms facilitates adverse influences on cardiac function in adult ventricular muscle due to desynchronized actin filament activation [58].

4. Sarcomeric cytoskeletal proteins

Cytoskeletal proteins contribute to cell shape, mechanical resistance, and morphological integrity of cardiomyocytes. The sarcomeric cytoskeletal proteins are referred to as titin, myosin binding protein C (C-protein), α-actinin, myomesin, M-protein, etc. [60]. In this review, we will only cover the isoform transitions of titin and myosin binding protein-C (C-protein) in cardiac disease.

4.1. Titin

Titin is a giant muscle protein expressed in vertebrate striated muscle. It is the third most abundant sarcomeric protein with an average about 10% of myofibril mass. It is the largest protein known to date, and is also known as connectin [61,62]. Titin spans the entire half of the sarcomere from Z-line to M-line, and two titin molecules form a continuous filament along the whole length of the sarcomere by overlapping at the M-line [4,5] (Fig. 1A). The three elements of titin in the extensible I-band region are key determinants of myocardial passive tension and play a critical role in elastic recoil of the cardiac myocytes and contribute to diastolic function during left ventricular (LV) filling phase [63]. These three elements are 1) tandem Ig segments consisting of serially-linked immunoglobulin (Ig)-like domains, 2) the spring-like PEVK segment (with high percentages of proline, glutamic acid, valine, and lysine), and 3) the N2B element with its extensible unique sequence (N2B-Us) [4,63,64].

Titin is encoded by a single gene and the alternative splicing of this single gene results in distinct isoform classes [64]. There are three major isoform classes in mammalian cardiac muscle [63]. These cardiac titin are the smaller, stiffer, adult N2B (3.0 MDa), the larger, more compliant adult N2BA (3.2–3.7 MDa) and the largest fetal cardiac titin (FCT: ~3.7 MDa) [4,64–66]. Alteration of the cardiac titin isoform expression ratios has recently been linked to cardiac disease. In animal models, a canine tachycardia-induced model of dilated cardiomyopathy (DCM) indicates increased N2B titin after two to four weeks of pacing [67]. Another study of spontaneously hypertensive rat model (SHR) also showed a reduced ratio of N2BA/N2B titin in response to pressure overload, consistent with elevated passive tension of heart [68]. In human patients, left ventricle biopsies from patients with diastolic heart failure (HF) had a reduced N2BA/N2B titin [69]. Chronically ischemic LVs of coronary-artery-disease (CAD) patients with congestive heart failure (HF) had nearly 50% N2BA titin (compared to total titin (N2BA + N2B)) while approximately 30% N2BA was found in the LVs of control donor patients [70]. Analysis of explanted nonischaemic human DCM hearts again demonstrated increased proportions of N2BA/N2B [71,72]. Upregulation of compliant isoforms has also been found in patients with heart failure with preserved ejection fraction (HFpEF), a group accounting for about half of all HF cases and characterized by increased diastolic stiffness [73,74]. Recently, a muscle-specific splicing factor, RNA binding motif 20 (Rbm20), has been found to regulate titin alternative splicing [75]. The Rbm20 deficient rats express largest titin isoform (~3.83 MDa), a most compliant titin isoform, and develop dilated cardiomyopathy [75–77], which has been confirmed by a most recent study with the Rbm20 knockout mice [78]. RBM20 mutations in patients with severe dilated cardiomyopathy also lead to the expression of the larger, compliant fetal cardiac titin isoform [75].

4.2. Myosin binding protein-C (C-protein)

Myosin binding protein-C (MyBP-C) is a thick filament-associated protein localized to the cross-bridge containing C zones of striated muscle sarcomeres, where it binds to myosin and titin. Titin fulfills the role of a molecular ruler [55], and MyBP-C plays a role as a spatially defined regulatory protein [56–59]. Myosin, MyBP-C, and titin form a stable ternary complex where MyBP-C is arranged regularly in 9 of the 11 thick-filament stripes [79].

Three isoforms of MyBP-C are known to exist in adult striated muscle. Skeletal muscle expresses at least two isoforms of MyBP-C. The fsMyBP-C is the fast skeletal isoform encoded by gene *MYBPC2* in human. The ssMyBP-C is the slow form in skeletal muscle encoded by *MYBPC1* in human [80]. Human cardiac MyBP-C (cMyBP-C) is encoded by gene *MYBPC3* [56]. The fast and slow skeletal muscle isoforms can be co-expressed in the same sarcomeres [81] and can coexist in variable ratios leading to diverse arrangements of the characteristic sarcomere stripes [82], so skeletal muscle has a great potential to adapt to alterations in MyBP-C isoforms by modification of co-expression ratios in a flexible way. However, the cardiac isoform is expressed only in cardiac muscle throughout development, and the cardiac isoform of MyBP-C cannot be trans-complemented by skeletal MyBP-Cs [83]. So far, to the best of our knowledge, there have been no reports of isoform transitions or altered ratios of skeletal and cardiac MyBP-C in failing heart. Nevertheless, mutation-caused truncated cardiac MyBP-C isoforms have been a cause of hypertrophic cardiomyopathy, which is beyond the scope of this review. More details can be obtained from recent reviews regarding mutations in cardiac myosin binding protein-C [84–88].

5. Conclusion and future perspective

The sarcomeric proteins myosin, actin, troponin, tropomyosin and titin show isoform switching during development. Some protein isoforms are due to distinct gene expression (myosin, actin, MyBPC, tropomyosin, TnI and TnC); others arise from alternative splicing (titin, tropomyosin and TnT). Aberrant sarcomeric protein isoform transitions and sarcomeric gene alternative splicing involved in the heart failure have been only partially identified. The isoform transitions associated with heart failure are mostly embryonic-like gene re-expression. The mechanism(s) of embryonic-like gene re-expression

may be attributed to isoform transitions with development. Therefore, the developmental mechanisms of isoform alterations of sarcomeric proteins are essential to decipher the embryonic-like gene re-expression in failing heart. However, the developmental mechanism of sarcomeric protein isoform transition is still poorly understood. Nevertheless, the understanding of protein isoform transitions in normal and failing heart is progressing rapidly. In the near future, we believe that manipulation of gene alternative splicing or ratios of isoform transitions of sarcomeric proteins may provide an encouraging strategy for the treatment of chronic heart failure.

Conflict of interest disclosures

None.

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