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The role of βII spectrin in cardiac health and disease

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Abstract

Spectrins are large, flexible proteins comprised of α-β dimers that are connected head-to-head to form the canonical heterotetrameric spectrin structure. Spectrins were initially believed to be exclusively found in human erythrocytic membrane and are highly conserved among different species. βII spectrin, the most common isoform of non-erythrocytic spectrin, is found in all nucleated cells and forms larger macromolecular complexes with ankyrins and actins. Not only is βII spectrin a central cytoskeletal scaflolding protein involved in preserving cell structure but it has also emerged as a critical protein required for distinct physiologic functions such as posttranslational localization of crucial membrane proteins and signal transduction. In the heart, βII spectrin plays a vital role in maintaining normal cardiac membrane excitability and proper cardiac development during embryogenesis. Mutations in βII spectrin genes have been strongly linked with the development of serious cardiac disorders such as congenital arrhythmias, heart failure, and possibly sudden cardiac death. This review focuses on our current knowledge of the role βII spectrin plays in the cardiovascular system in health and disease and the potential future clinical implications.

Keywords

Spectrin; βII spectrin; Ankyrin; Cytoskeleton

1. Introduction

The spectrin family of proteins was first discovered in 1968 by Marchesi and Steers [1] as components of the human erythrocytic membrane [2], and was initially thought to be exclusively present in red blood cells [3,4]. While many subsequent experimental attempts failed to demonstrate the presence of spectrin in various non-erythroid cells [3,4], notable discoveries in brain did identify new peptides with calmodulin and actin binding properties

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Conflict of interest statement

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comprised of 2 subunits (≈ 240 and 235 kDa) and were described as '*Fodrin*' [5], '*brain actin-binding protein*' [6] and '*Calspectin*' [7]. No formal relationship was established between spectrin and these newly discovered brain peptides, yet these new proteins (yet to be termed spectrin) consistently had the same immunological, structural and functional degree of similarity to erythrocyte spectrin [8]. In 1981, Goodman and colleagues discovered that proteins with spectrin-like properties were potentially present in different cells and tissues such as brain, kidney, skeletal muscle, lens, small and large intestines and cardiac muscle [5,8–12]. Non-erythroid spectrins emerged as novel large actin-associated cytoskeletal proteins [2] that maintained cell shape and integrity and formed larger molecular complexes with ankyrins [13].

βII spectrin (the most common member of non-erythroid spectrins) [14] has emerged as a key cytoskeletal protein that is part of a larger macromolecular complex involved in diverse physiologic functions. βII spectrin is critical in posttranslational targeting and localization of essential membrane proteins [15–17], plays a prominent role in signal transduction [18,19], and notably serves as a major scaflolding protein [2]. Defects in βII spectrin have been associated with serious cardiac pathologies such as congenital arrhythmia, acquired and congenital forms of heart failure, and possibly sudden cardiac death [20–23]. This review focuses on the recent advances in defining the role of βII spectrin in the cardiovascular system in physiologic and pathologic states.

2. The spectrin genes and nomenclature

The nomenclature of spectrins has undergone several iterations. Winkelmann and Forget [24] classified spectrins by Roman numerals in the order of their characterization while subtypes were denoted by the Greek symbol sigma (Σ: capital letter, σ: small letter) followed by Arabic numbers.

Spectrin proteins are expressed from numerous genes in metazoans. There are seven genes that code for spectrins in mammalian organisms compared to only three genes in invertebrates. Unlike the three spectrins in invertebrates, the mammalian genome contains two α and five β spectrin genes named in the order of their discovery. *SPTA1* gene [25] codes for αI that is expressed in erythroid cells, while *SPTAN1* [26] codes for at least four and possibly up to eight different αII isotypes that are present in all non-erythroid cells [27]. The conventional βI-IV-spectrins are encoded by *SPTB, SPTBN1, SPTBN2* and *SPTBN4*, respectively and *SPTBN5* encodes a heavy βV-spectrin [2,28]. βI spectrin is the only form expressed in erythrocytes. The spectrin product of these genes can be modiffed via extensive alternative processing of pre-mRNA giving rise to a wide diversity of spectrin spliceoforms. This is particularly important with respect to regulating the interactive and modulatory characteristics of spectrins [2,26–29].

3. Structural domains of spectrin

Spectrins, believed to have evolved from α-actinin [30–33], are formed of two large, similar but non-identical subunits, termed α and β [2,30–36]. Spectrins are flexible rods that have a contour length of approximately 200–260 nm with an actin-binding domain (ABD) on each

end [2,37–39]. The α and β subunits are connected side-by-side in an antiparallel fashion via hydrophobic interactions supplemented by electrostatic forces of attraction [40,41] to form a heterodimer [2,28,31,39–41]. This involves an interaction between two repeats near the NH_2-terminus of one α spectrin chain and the COOH-terminal region of the antiparallel β subunit. Each of the 2 corresponding dimers is then assembled head-to-head via partial repeats in both α and β subunits to form the final heterotetramer structure of spectrin [2,28,31,42,43]. Owing to the high affinity between α and β chains, spectrins primarily exist as heterotetramers rather than autonomous α or β subunits.

The canonical spectrin subunit is highly conserved among species [2,28], and is comprised of successive repeats of 106 amino acid residues termed spectrin repeats that are folded in a triple α-helical coiled structure. This structural form and interconnection of spectrin repeats are believed to play a role in the flexibility of spectrins [44]. The α and β subunits are comprised of 21 and 17 repeats, respectively [31]. The only exception is βV spectrin which has 30 repeats [45]. The last repeat in each spectrin is an incomplete repeat that mediates end-to-end association between one helix of α spectrin and two helices of β spectrin to form a triple helical bundle [46].

βII spectrin, like all conventional β spectrins, contains 2 tandem calponin homology (CH_1 & CH_2) domains which both comprise an actin-binding domain (ABD) at the amino-terminal [2,28,31,47,48]. Linked to the ABD domain of βII subunit are 17 successive triple helical motifs and terminates with a carboxyl region. The COOH-terminal region of the 14th repeat and the entire 15th repeat are a prerequisite for ankyrin binding [46,49]. The carboxyl-terminal of βII spectrin is differentially spliced giving origin to long and short βII isoforms (βIIΣ1 and βIIΣ2 respectively) [31,50,51]. The long carboxyl terminus of the last partial repeat of βII spectrin is linked to a pleckstrin homology (PH) domain [50]. The spliced βII isoforms with short C-terminal regions lack this PH domain [52]. The PH domain, a seven stranded antiparallel β-sheet, is comprised of approximately 100–120 amino acids and is located approximately 50–60 amino acid residues before the end of the C-terminus of β chain. This domain serves as a ligand binding site for many phospholipids involved in signal transduction [28,50,53–56]. Immunofluorescence staining shows that βII spectrin is localized in a striated pattern in isolated mouse myocytes [20].

The alternatively spliced short variants of βII spectrin called *ELF* (embryonic liver fodrin) share some similarities with the long βII isoform. *ELF*-3, a 200 kDa β spectrin and the longest form among other *ELFs*, is a short βII isoform with an ABD, a 17 repeat domain and COOH terminal lacking the PH domain [52]. On the contrary, *ELF*-1, a 27 kDa β spectrin, shares no degree of homology in domain 2 of the long βII isoform [57], but has a sole CH_1 domain similar to that of the other β spectrins and a C-terminus similar to the short βII isoform [2,52,57].

4. Role of βII spectrin in the heart

The cardiac cytoskeleton has recently emerged as a crucial player for maintaining the cardiac membrane integrity with respect to structure and function in physiologic and pathologic states. Disorders in cardiac cytoskeletal components have been strongly

associated with cardiac myopathies, dystrophies, aortopathies and electrical conduction abnormalities [20,58–61]. While βII spectrin has a prominent role in many organ systems (Table 1), it has also emerged as a pivotal protein in maintaining normal cardiac membrane excitability, mechanical function [20] and proper embryogenesis [62].

5. Role in embryonic heart development

Emerging data suggests that βII spectrin plays an important role in embryonic heart development [62]. Data in mice demonstrate that complete deletion of βII spectrin results in intrauterine death with multiple defects including hepatic, neural, gastrointestinal and angiogenesis abnormalities [77]. Notably loss of βII spectrin is a possible cause for the development of congenital heart defects [62,78,79]. Furthermore, a study performed on homozygous mutant embryos for βII spectrin gene demonstrated that there was a significant difference in heart size between the wild-type embryos and the homozygous mutant embryos with the latter having smaller heart size at embryonic day 15.5 (E15.5). Further histologic studies revealed failure of ventricular wall thickening and blood vessel formation in the homozygous mutant group [62]. Moreover, the embryonic cardiomyocytes of βII spectrin conditional knockout mice displayed an aberrant distribution of tropomyosin and a significant down-regulation in the expression of α-smooth muscle actin (α-SMA), cardiac homeobox protein (NKx2.5) and dystrophin [80] which are muscle differentiation markers [62]. These defects subsequently have an s adverse effect on the contractile ability of cardiomyocytes in vivo. In addition, loss of βII spectrin in homozygous mutant embryos interferes with cardiac cell differentiation and induces extensive apoptosis at E16.5 [62]. It is noteworthy that genetic mutations in dystrophin leads to congenital muscular dystrophies such as Duchenne muscular dystrophy and Becker muscular dystrophy, and many of these patients develop dilated cardiomyopathy and ventricular arrhythmias [81,82]. Hence the aforementioned data strongly suggests that βII spectrin is critical for proper cardiac development.

6. Role in cardiac membrane excitability

Until recently, arrhythmogenic cardiomyopathies were mainly linked to disorders in ion channels [83], however, little was known about arrhythmia resulting from defects in cytoskeletal-associated proteins [20]. Recent data reveal that defects in the βII spectrin-based cytoskeleton disrupt normal electrical conduction in the heart [20], potentially leading to congenital as well as acquired human arrhythmia [21].

Recent data has shown that at the T-tubule in cardiac myocytes, the αII/βII tetramer (linked to actin) recruits ankyrin-B [18] and associates via its ankyrin-binding domain with the NH2 terminal ZU5 ($Zu5^{N}$) domain of ankyrin-B [84]. This molecular complex interacts with other membrane-associated proteins such as Na^{+}-K^{+}-*ATPase* and Na^{+}-Ca^{2+}exchanger (known ankyrin-binding partners) and is important for their localization [20]. A newly identified human variant of ankyrin-B showed a substantial decrease in binding with βII spectrin in heart [20]. Using primary neonatal cardiomyocytes of ankyrin-B conditional knockout (cKO) mice the authors were able to elucidate the relationship between βII spectrin and ankyrin-B, and demonstrated that after transfection the human variant failed to

localize Na^{+}-Ca^{2+} exchanger when compared with the wild-type ankyrin-B which rescued Na^{+}-Ca^{2+} ion channel localization [20]. Notably previously identified ankyrin-B variants (DAR976AAA and A1000P) [18] which also lacked spectrin-binding activity also showed similar results. Together these data demonstrate that βII spectrin and ankyrin-B are molecular partners in heart, and importantly they form an important macromolecular complex. Interestingly, while ankyrin-B is critical for βII spectrin targeting in the neonatal period [18], βII spectrin plays the predominant role in the mature cells and is crucial for proper ankyrin-B expression and localization [20]. Consistent with these findings, βII spectrin protein expression in right atrial samples of patients with atrial fibrillation (AF) was significantly decreased compared with patients in sinus rhythm [85–87]. Likewise, patients with loss-of-function genetic mutations in ankyrin-B also developed AF. The ankyrin-B protein levels in these patients were markedly decreased in their right atria as well [85]. This is consistent with the recently identified congenital human arrhythmia that arose due to a dysregulation in the ankyrin-B/βII spectrin pathway [20].

Furthermore, telemetry studies performed on cardiac-specific βII spectrin knockout mice showed aberrant cardiomyocyte electrical activity [20]. ECG readings of these mice showed bradycardia, atrioventricular nodal block, and increased rate variability. Notably, pro-arrhythmic patterns were observed, such as widened QRS complexes and prolonged QT interval at baseline and ventricular arrhythmia and mortality was observed in several animals after catecholaminergic induced stress. However, there were no notable differences between βII spectrin knockout mice and the wild-type littermates in terms of cardiac output and left ventricular ejection fraction [20].

7. Role in heart failure and cardiac remodeling

Dysregulation of βII spectrin has been linked with the development of acquired forms of heart failure (HF) in both human and animal models [21]. Following transverse aortic constriction (TAC), βII spectrin cKO mice suffered severe and accelerated cardiac failure after 6 weeks characterized by left ventricular free wall degeneration and interventricular septal vacuolation. Furthermore, βII spectrin levels were found to be significantly decreased in the left ventricular tissues of ischemic and non-ischemic heart failure in murine and canine myocardial failure models [21].

It is critical to note that abnormal and selective cardiac remodeling occurs with the loss of βII spectrin in heart [20]. In accordance with earlier studies in avian erythrocytes (whose erythrocytic spectrin is more similar to mammalian non-erythroid spectrin [24,88,89]) where α spectrin levels were decreased in the absence of erythrocytic β spectrin [90–92], βII spectrin cKO mice also displayed a sharp decline in αII levels in the heart [20] (Table 2). The levels of select proteins in the heart, namely βI spectrin, α and β tubulin, were all upregulated in βII spectrin cKO mice, likely as a compensatory process [20]. These data are consistent with previous reports of elevated tubulin expression and remodeling in failing myocardium [93]. The levels of actin and desmin proteins in βII spectrin cKO mice were not altered which further support that the remodeling process is selective [20].

In human heart failure the levels of βII spectrin protein were significantly decreased in patients with non-ischemic [21] and ischemic heart failure [94], as were the levels of ankyrin-B protein [21,94] and *Na^+-K^+-ATPase* [94]. Contrarily, the levels of βII spectrin mRNA transcripts as well as those of ankyrin-B and *Na^+-K^+-ATPase* were not dramatically different between diseased and normal groups [21,94]. This observation is likely due to post-translational process induced by Ca^{2+}- and calpain-dependent proteolysis [21]. In avian erythrocytes, remodeling at the cellular level is associated with spectrin-based cytoskeletal cleavage [90–92], and this proteolytic process is induced by calpains [95–97]. Similarly, the heart and brain of adult mice showed that cleavage of the βII spectrin based cytoskeleton occurs following exposure to pathological concentrations of Ca^{2+} [21]. It is important to note that the degradation of βII spectrin and ankyrin-B was prevented by the calpain inhibitor MDL-28170 [21,95]. In addition, previous data show that reactive oxygen species (ROS) has been linked with apoptosis of cardiomyocytes through various signaling pathways including activation of cardiac calpain proteases [21,98,99], and this is another mechanism that could lead to breakdown of the spectrin cytoskeleton. As the failing heart exhibits dysregulation in Ca^{2+} homeostasis and a rise in the concentration of ROS, this likely [100] explains decreased protein expression of βII spectrin and ankyrin-B proteins in non-ischemic heart failure patients [21].

8. Future directions and clinical implications

8.1. New therapeutic targets

Several molecules underlying biogenesis and regulation of the ryanodine receptor 2 (RyR_2) have been identified, including calstabin, calmodulin, protein kinase A (PKA), and junctophilin to name a few [103–105]. Unfortunately there has been a failure of past ion channel specific therapies for heart disease—hence it is critical that we find new molecules that can be targeted to regulate contractility/excitability beyond ion channels and receptors, and the calcium-induced calcium release (CIRC) pathway is an ideal target. As the cytoskeletal protein βII spectrin is a critical node linked to local RyR_2 regulation [20], it might be exploited as a potential diagnostic and therapeutic target for heart failure and arrhythmia.

It is well known that mislocalization or dysfunction of RyR_2 is associated with the development of arrhythmia, heart failure and sudden cardiac death in animals and humans [105–110]. In βII spectrin cKO mice, the levels of RyR_2, *Na^+-Ca^{2+}* exchanger and *Na^+-K^+-ATPase* were dramatically decreased (Table 2) [20]. There were no differences observed in T-tubule L-type calcium channel localization or T-tubule morphology between wild type and βII spectrin cKO mice, but there were heterogeneity, reduced size and intensity of the remaining RyR_2 clusters [20]. In addition, the ventricular cardiac cells of βII spectrin cKO mice exhibited recurrent spontaneous *Ca^{2+}*-induced after-depolarizations (Fig. 1). These afterdepolarizations were eliminated with ryanodine which is known to inhibit *Ca^{2+}* release. Compared with cardiac cells of the wild-type mice, the βII spectrin cKO mice showed almost a threefold increase in spontaneous *Ca^{2+}* waves that are known to provoke arrhythmia [20]. It is also noteworthy that βII spectrin controls RyR_2 localization independently of ankyrin-B as studies with ankyrin-B haploinsufficient mice showed no

abnormalities in RyR_2 localization [111]. Thus, βII spectrin plays an integral role in properly targeting and localizing important cardiac membrane-associated proteins, and abnormalities with spectrin localization could lead to arrhythmias induced by calcium overload.

As previously mentioned, dysregulation in Ca^{2+} homeostasis and subsequent activation of calpain enzymes occur in heart failure and consequently βII spectrin-based cytoskeleton degradation and remodeling ensue. This degradation was prevented by the calpain inhibitor MDL-28170 [20]. Thus, calpain inhibition could serve as a future therapeutic intervention to prevent destabilization of the spectrin cytoskeleton. As βII spectrin-based cytoskeletal cleavage and cellular remodeling are tightly linked with Ca^{2+}-dependent calpain proteases, it is reasonable to hypothesize that malignant proteolysis of βII spectrin plays a direct role in local RyR_2 regulation, hence calpain inhibition could have a role in preserving RyR_2 integrity. Further research aimed at unveiling the complexity of calpain substrate specificity and the mechanisms involved in off- and on-target effects of calpain inhibitors is needed.

8.2. Breakdown products and biomarkers

Spectrin breakdown products (SBDPs) are produced by calpain- and caspase-3-mediated mechanisms and can be detected in the cerebrospinal fluid (CSF) [112–114] and serum [115]. In brain, αII spectrin is degraded by calpain into 2 main fragments (150 kDa and 145 kDa) while caspase-3-induced proteolysis results in the formation of a 150 kDa fragment which is further degraded yielding a 120 kDa spectrin breakdown product [116–120]. Caspase-3-mediated proteolysis is believed to be an indication of apoptosis, while calpain-induced proteolytic cleavage is proposed to be an indicator of excitotoxic and necrotic neuronal death [116–122]. In rat models, four main βII spectrin degradation products (110, 108, 85, 80 kDa) accumulated in the cortical cells due to activation of calpain-2 and caspase-3 following brain injury. The 110 kDa and 85 kDa fragments are calpain specific BDPs while the 108 kDa and 80 kDa are caspase specific products [119,123]. These recent reports showed that both αII and βII SBDPs could serve as potential biomarkers that would aid in the clinical diagnosis and outcome as well as correlate with the severity of neurologic insults [113,114,124] including traumatic and ischemic brain injuries and neurodegenerative diseases [120] such as Alzheimer's Dementia. Likewise, it has been demonstrated in the heart of adult mice that cleavage of the βII spectrin based cytoskeleton occurs following Ca^{2+}-triggered calpain activation (Fig. 2) [21]. One could hypothesize that βII spectrin breakdown products could serve as an early diagnostic biomarker for acute cardiovascular diseases as cardiac and myocyte insult typically leads to calcium release and ROS activation, which inevitably will lead to cleavage of spectrins [21].

Cardiovascular disease remains the number one cause of death in the U.S. Further, dilated cardiomyopathies, HF and arrhythmias are a significant health burden. Despite advancement in medical therapies, cardiac resynchronization therapies, and left ventricular assist devices, HF remains a global epidemic with most deaths ultimately caused by arrhythmias [125]. Additional insight and research into the molecular underpinnings of the cardiac cytoskeleton could lead to new diagnostic and therapeutic targets for therapies and warrants further investigation.

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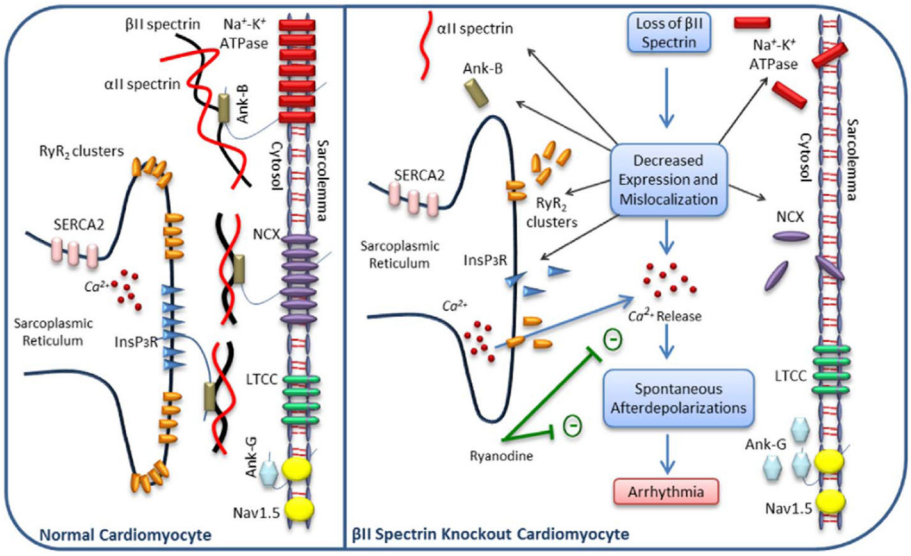

Fig. 1.

A schematic view of a normal cardiomyocyte and a βII spectrin knockout cardiomyocyte. In the absence of βII spectrin protein, the expression levels of ryanodine receptor 2 (RyR_2), Na^+-K^+-ATPase and Na^+-Ca^{2+}-exchanger (NCX), Ankyrin-B (Ank-B), αII spectrin are significantly decreased and showed defective localization [20]. The expression of both sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2) and voltage-gated Na^+ channel (Nav1.5) are not impacted by the loss of βII spectrin [20]. Additionally, both SERCA2 and L-Type Ca^{2+} channel (LTCC) are properly localized. The levels of Ankyrin-G (Ank-G) are increased in the absence of βII spectrin, likely as a compensatory response to decrease ankyrin-B expression [20]. Defects in ryanodine receptors result in aberrant Ca^{2+}-dependent release and subsequent spontaneous afterdepolarizations which eventually lead to the development of arrhythmias. In a murine model of a cKO of βII spectrin, ryanodine was shown to inhibit Ca^{2+} release and abolishes these spontaneous afterdepolarizations. It is important to note that further research is warranted to demonstrate the localization of Nav1.5 and the expression of LTCC in βII spectrin deficiency. In addition, no data are currently available that directly links the expression and localization of inositol triphosphate receptor ($InsP_3R$), a known ankyrin-B binder, to βII spectrin protein. However, mislocalization and decreased expression of $InsP_3R$ is expected due to decreased ankyrin-B expression [101,102].

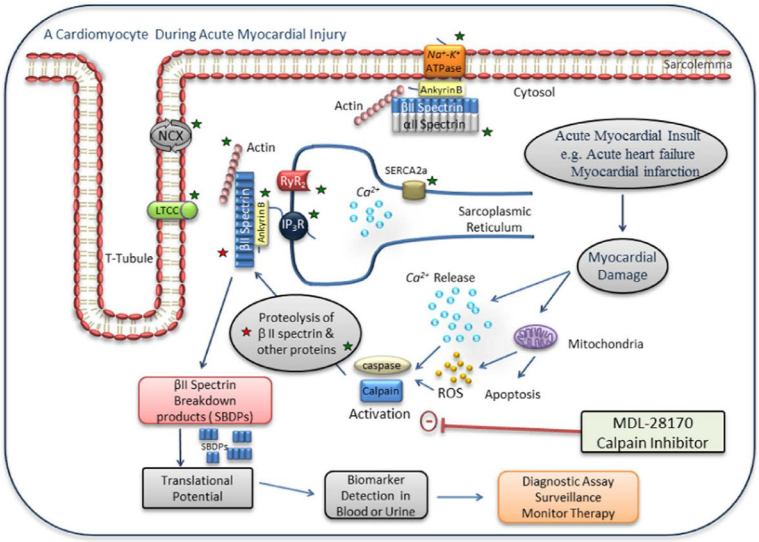

Fig. 2.

A schematic view of a cardiomyocyte during acute myocardial injury. In the event of acute myocardial damage, Ca^{2+} dysregulation occurs leading to Ca^{2+} leak from the sarcoplasmic reticulum and subsequent activation of calpain and caspase enzymes. Increased levels of reactive oxygen species (ROS) released within the cardiomyocyte also activate Ca^{2+}dependent proteases. These proteases then cleave βII spectrin (denoted by red star) and other potential protein substrates (denoted by green stars) producing βII spectrin breakdown products (SBDPs) which could have translational potential as a biomarker of cardiac damage via detection in blood or urine. SBDPs could serve as early biomarkers in acute cardiac injury that would aid in the diagnosis, surveillance, and monitoring of patients who have acute cardiac syndromes. Of note the calpain inhibitor MDL-28170 prevents the degradation of βII spectrin, and this could serve as a therapeutic intervention for the stabilization of βII spectrin following acute cardiac injury [20]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

The physiologic roles of βII spectrin in different organ systems.

Organ system	Physiologic roles of βII spectrin	Ref.
Pulmonary system	De novo synthesis and stabilization of lateral membrane of bronchial epithelial cells.	[17]
	Maintaining polarity of E-cadherin and *Na^+-K^+-ATPase.*	[16,63,64]
Nervous system	Binding to small synaptic vesicles via synapsin-I involved in neurotransmission.	[65,66]
	Important component of paranodal junctions involved in saltatory conduction.	[67,68]
	Maintaining structural integrity of neurons.	[68,69]
	Molecular partner to α-synuclein that regulate neurite growth during synaptogenesis.	[70]
Hepatic system	Hepatocellular carcinoma suppression mainly via serving as an adaptor protein for Smad3 and Smad4 involved in TGF-β signaling pathway.	[19,71]
	Involved in the regenerative process following partial hepatectomy.	[72–74]
	A mediator and effector protein in acetaminophen-induced liver injury.	[14]
	ELF-3 has a role in intrahepatic bile duct formation and hepatic cells differentiation and polarization	[52]
Renal system	Maintaining polarity of *Na^+-K^+-ATPase* in renal tubular cells	[75,76]

Table 2

Cardiac expression and localization of different proteins linked to βII spectrin loss.

Protein	Expression levels	Proper localization	Ref.
αII spectrin	↓	No	[20]
βI spectrin	↑	Not available	[20]
Actin	–	Yes	[20]
Desmin	–	Yes	[20]
α tubulin	↑	Not available	[20]
β tubulin	↑	Not available	[20]
Ankyrin-B	↓	No	[20]
Ankyrin-G	↑	Not available	[20]
Ankyrin-R	–	Not available	[20]
Ryanodine receptor 2	↓	No	[20]
Na^+-K^+-ATPase	↓	No	[20,101]
Na^+-Ca^{2+}-exchanger	↓	No	[20,101]
Inositol triphosphate receptor	↓	No	[101,102]
L-type calcium channel (Cav1.2)	Not available	Yes	[20]
Voltage-gated Na channel (Nav1.5)	–	Not available	[20]
Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2)	–	Yes	[20]