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Review Article

S100A1: A Regulator of Striated Muscle Sarcoplasmic Reticulum Ca^{2+} Handling, Sarcomeric, and Mitochondrial Function

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Calcium (Ca^{2+}) signaling plays a key role in a wide range of physiological functions including control of cardiac and skeletal muscle performance. To assure a precise coordination of both temporally and spatially transduction of intracellular Ca^{2+} oscillations to downstream signaling networks and target operations, Ca^{2+} cycling regulation in muscle tissue is conducted by a plethora of diverse molecules. Ca^{2+} S100A1 is a member of the Ca^{2+}-binding S100 protein family and represents the most abundant S100 isoform in cardiac and skeletal muscle. Early studies revealed distinct expression patterns of S100A1 in healthy and diseased cardiac tissue from animal models and humans. Further elaborate investigations uncovered S100A1 protein as a basic requirement for striated muscle Ca^{2+} handling integrity. S100A1 is a critical regulator of cardiomyocyte Ca^{2+} cycling and contractile performance. S100A1-mediated inotropy unfolds independent and on top of βAR-stimulated contractility with unchanged βAR downstream signaling. S100A1 has further been detected at different sites within the cardiac sarcomere indicating potential roles in myofilament function. More recently, a study reported a mitochondrial location of S100A1 in cardiomyocytes. Additionally, normalizing the level of S100A1 protein by means of viral cardiac gene transfer in animal heart failure models resulted in a disrupted progression towards cardiac failure and enhanced survival. This brief review is confined to the physiological and pathophysiological relevance of S100A1 in cardiac and skeletal muscle Ca^{2+} handling with a particular focus on its potential as a molecular target for future therapeutic interventions.

1. Introduction

Ca^{2+} is a highly versatile intracellular signal that controls many different cellular functions. Therefore, Ca^{2+} signals need to be flexible yet precisely regulated. Besides controlling gene transcription and growth, Ca^{2+} regulates the contraction and relaxation in muscles tissue. Skeletal and cardiac muscle belong to the striated tissue and share many functional aspects. The Ca^{2+} signals require downstream proteins to connect Ca^{2+} oscillations to different signaling domains. Ca^{2+} cycling in muscle tissue is regulated by a plethora of proteins, transmitting the Ca^{2+} messages with precision and in a temporally and spatially coordinated manner. One of these specific Ca^{2+} binding protein families within muscle cells is the S100 protein family.

The process of excitation-contraction coupling (ECC) in skeletal and cardiac muscle cells requires membrane depolarization. After membrane depolarization, Ca^{2+} influx is activated via voltage-gated L-type Ca^{2+} channels into the cytosol of both skeletal muscle cells and cardiac myocytes [1]. This rise in cytoplasmic Ca^{2+} concentration leads to Ca^{2+} release from the sarcoplasmic reticulum (SR) (Ca^{2+} induced Ca^{2+} release-CICR) by activation of Ryanodine receptors (RyR), whereas in skeletal muscle voltage dependent Ca^{2+} release occurs (VICR). After Ca^{2+} release of the RyR, Ca^{2+} molecules subsequently bind to the contractile proteins such as troponin c, which causes contraction of the myocytes. Thereafter, Ca^{2+} is cleared from the cytosol by reuptake of Ca^{2+} into the SR by the action of a sarcoplasmic reticulum Ca^{2+} ATPase (SERCA).

Much evidence has accumulated that abnormal regulation of intracellular Ca^{2+} by the SR plays a key role in the development of cardiac diseases. A decreased Ca^{2+} transient leads to a decreased contractility of the cardiomyocytes. The reduced Ca^{2+} transients are mainly related to a decreased SR Ca^{2+} load. Correction of the disorder of Ca^{2+} cycling has a potential as a new and intriguing therapeutic strategy against cardiac disease states.

Among others proteins, it has been proven that S100A1 regulates sarcoplasmic reticulum Ca^{2+} handling in skeletal and cardiac muscles [2, 3]. In this brief review, we discuss the physiological role of S100A1 in the regulation of ECC in skeletal and cardiac muscles. In addition, we focus on pathophysiological consequences of altered S100A1 expression in cardiovascular disease to provide a comprehensive understanding of the role of S100A1. Finally, we aim to highlight the potential of an S100A1-targeted therapy in cardiovascular diseases.

2. S100 Protein Family

S100 Ca^{2+}-binding proteins are the largest subfamily of EF-hand proteins (25 human genes), putatively targeting more than 90 proteins (previously reviewed in [4, 5]). S100 proteins are small acidic proteins (10–12 kDa) that are found exclusively in vertebrates. These proteins were first identified by Moore in 1965 and characterized as "S100" in consequence for their unique solubility in 100% saturated solution with ammonium sulphate and were designated by consecutive arabic numbers placed behind the stem symbol, for example, *s100a1, s100a2, s100a3*, and so forth [4] due to their clustered organization on human chromosome 1q21.

Each S100 monomer contains two EF-hand Ca^{2+} binding domains interconnected by an intermediate region (hinge region) (Figure 1) [6] exerting unique features when compared with other EF-hand proteins. First, the two EF hands in each monomer differ in sequence and affinity for Ca^{2+} binding. The c-terminal EF hand contains the classical canonical Ca^{2+} binding motif and ligates Ca^{2+} in a similar manner to calmodulin and troponin C, resulting in K*d* 10–50 μM. The NH2-terminal pseudo-EF hand contains two additional amino acids and is a characteristic of S100 proteins. Consequently, Ca^{2+} affinities decreased to a K*d* between 200–500 μM (reviewed in [6]). Importantly, Ca^{2+} affinity is regulated by posttranslational mechanism facilitating Ca^{2+} binding and activation of S100 proteins even at nanomolar free Ca^{2+} concentrations. Ca^{2+} binding eventually triggers exposure of a hydrophobic epitope which is believed to mediately represent a major interaction site enabling accommodation of target proteins through the exposure of a hydrophobic epitope that surface represents the interaction site of most S100 proteins for the binding of target proteins (Figure 2). The second unique characteristic compared to EF-hand proteins of S100 proteins is their dimeric nature, as it has been shown both in vivo and in vitro that S100 proteins form noncovalent homo- and heterodimers (Figure 2). Third, S100 proteins are expressed in a tissue- and cell- specific fashion, pointing to higher degree of specification. The most abundant S100 protein isoform in cardiac and skeletal muscle is S100A1 among other S100 isoforms such as S100A4, S100A6, and S100B and in heart S100A1 is mainly found in ventricular cardiomyoctes.

3. S100A1—A Member of the S100 Protein Family

In a wide range of small (mouse, rat) and large (rabbit, dog, swine) animals, S100A1 is preferentially found in the heart and in lower concentrations in skeletal muscle [8–10]. Healthy human tissue exhibits a similar distribution [11], and most recently, large-scale analyses of the human transcriptome by Su et al. [12] and Shmueli et al. [13] comprehensively confirmed that the human heart is the predominant location of S100A1. S100A1 expression steadily increases during cardiac development in mice and reaches a plateau in ventricular myocardium in the postnatal state [14]. Estimations of total intracellular concentrations of S100A1 in isolated murine and rat left ventricular myocytes revealed a range between 40 to 200 nM ([12], Völkers M, unpublished observations).

In the adult heart, S100A1 protein is not uniformly expressed but exhibits its highest mRNA and protein levels in the left ventricle, with lower concentrations in the right ventricle and atria [6, 15]. Analysis of the S100A1 promoter in rodents identified a subset of transcription-factor consensus sequences (i.e., homeobox protein NK-2 homolog E, myocyte enhancer factor-2, GATA4) [14] that are well known to drive cardiac-specific gene expression of cardiac troponin C or calsequestrin and potentially to convey predominant cardiac expression of S100A1. First clinical interest in S100A1 has been sparked due to its altered expression in diseased myocardium.

3.1. Protein Structure. The S100A1 monomer with a molecular weight of 10.4 kDa consists of two EF-hand Ca^{2+} binding motif connected by a hinge region. In each EF-hand, a Ca^{2+} binding loop is flanked by α-helices, resulting in a helix-EF-hand-helix arrangement. Helix 1 and II flank the n-terminal loop, whereas helices III and IV flank the c-terminal loop (Figure 1). Stabilization of the homodimer occurs independent of Ca^{2+} binding through hydrophobic bonds between helices I and I' of each monomer [6]. After Ca^{2+} binding each monomer undergoes a conformational change resulting in the exposure of a hydrophobic pocket in the C-terminus which is believed to be the major docking site for Ca^{2+}-dependent interactions between S100A1 and its target proteins. This pocket consists of residues of the hinge region, helix III and the C-terminus. Since residues within the hinge region and C-terminus display the greatest sequence variability among S100 isoforms, they are viewed to mediate isoform-specific target recognition [6]. Ca^{2+} binding to individual EF-hands in the S100A1 dimer has been estimated to occur with a K*d* of 200–500 μM at the N-terminal and a K*d* of 10–50 μM at the C-terminal. However, Ca^{2+} affinity of both sides is tightly regulated by

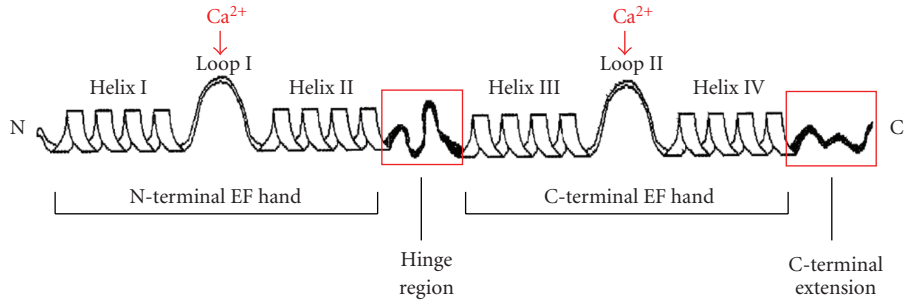

Figure 1: Schematic depiction of the secondary structure of an S100 protein. The monomeric structure consists of a repetitive EF-hand motif, whereas eachCa^{2+}-binding Loop (Loop I and II) is flanked by α-helices. The N-terminal and the C-terminal EF hands are connected by a linker region (hinge region). The hinge region and the C-terminal extension (boxed in red) display the least amount of sequence homology among S100 paralogs. Reproduced with modifications from Donato [5]. [http://www.ncbi.nlm.nih.gov/pubmed/11390274?itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum&ordinalpos=9].

posttranslational mechanism, that is, redox dependent S-glutathionylation of cysteine residues which enables S100A1 to sense Ca^{2+} even at nanomolar concentration (see below).

3.2. Cardiac Subcellular S100A1 Location. Dependent on the Ca^{2+} binding in adult cardiomyocytes, S100A1 mainly resides on the SR, mitochondria and myofilaments. At the molecular level, S100A1 has been shown to interact with the cardiac ryanodine receptor (RyR2) [16–19], the SR Ca^{2+}-ATPase 2a (SERCA2a)-phospholamban (PLB) complex [16, 20, 21], the F1 portion of the mitochondrial ATP synthase [22] mostly in a Ca^{2+}-dependent manner and acts also as a binding partner for the giant myofilament protein titin [23, 24]. As mentioned, Ca^{2+} binding triggers exposure of hydrophobic epitope mainly dened by residues within the S100A1 hinge region and COOH-terminal extension (Figure 2) [7]. Because most actions of S100A1 apparently rely on transition to its Ca^{2+}-activated state, it is important to note that recent studies provided first evidence for redox-dependent posttranslational control of S100A1's Ca^{2+} sensitivity. NO-dependent S-glutathionylation of a single cysteine residue within the hydrophobic COOH terminus eventually facilitated binding of Ca^{2+} at nanomolar concentrations reflecting diastolic Ca^{2+} levels in cardiomyocytes [25, 26]. The only S-nitrosylation site of S100A1 is its unique Cys-85 residue. Recent studies indicate that under physiological conditions the ability of S100A1 protein to act as a calcium receptor can be turned on by glutathionylation (cysteinylation) of its Cys85 residue and off by reduction of the mixed disulfide S100A1–glutathione (S100A1–cysteine) species. To our knowledge no studies in cardiomyocytes exist which investigate the basal redox state of S100A1. However preliminary data indicate that the addition of reducing agents to the cardiomyocytes abolished the inotropic effects of S100A1 in vitro (M. Volkers, unpublished data).

Redox and NO-related posttranslational modification of single cysteine residue might therefore enable S100A1 to sense spatially defined short-term as well as long-term global Ca^{2+} oscillations in cardiac cells over a broad concentration range enabling its interaction with target proteins such as RyR and SERCA. However, the impact of altered redox conditions and NO homeostasis on S100A1 actions in myocardium has not yet been tested.

4. Molecular Targets and Physiological Functions in Cardiac Myocytes

4.1. Lesson from Genetically Altered Miced. Gain- and loss-in-function studies comprehensively characterized S100A1 as a unique molecular inotrope in vivo and ex vivo. Increasing S100A1 protein levels in isolated adult and neonatal cardiomyocytes enhanced both their systolic and diastolic performance through modulation of cellular Ca^{2+} handling and myofilament function [17, 19, 20, 24, 27]. Cardiac-specific overexpression of S100A1 in mice displayed a phenotype of chronically heightened cardiac performance without detrimental effects on survival or development of cardiac hypertrophy [19]. The S100A1-mediated increase in cardiac performance is independent of beta-adrenergic stimulation and is even preserved under stimulation with catecholamines. Expression analysis yielded that S100A1-mediated inotropic effects are neither the result of altered abundance of SR and sarcolemmmal Ca^{2+} regulators, respectively, nor that they depend on β-AR signalling [2, 15, 17–19, 21, 28–35]. To our knowledge, the apparent independence of S100A1 inotropic actions from the β-AR signalling pathway is a unique feature of the Ca^{2+} sensing protein and of particular importance in cardiovascular physiology and therapy.

In line with cardiac specific overexpression of S100A1 in transgenic mice, viral-based overexpression of S100A1 in rabbit and rat hearts and isolated cardiomyocytes recapitulated the hypercontractile phenotype based on enhanced Ca^{2+} cycling but independent, and additive to beta-AR signalling [15, 17, 18, 33, 34]. Therefore, it is interesting to note that acute or chronic elevated S100A1 proteins in cardiomyocytes induce sustained cardiac inotropy, without toxic effects to the cardiomyocytes. This is clinically important because chronic inotropic stimulation of the heart through activation of the beta-receptors or downstream effectors such as PKA eventually led to cardiac hypertrophy and failure.

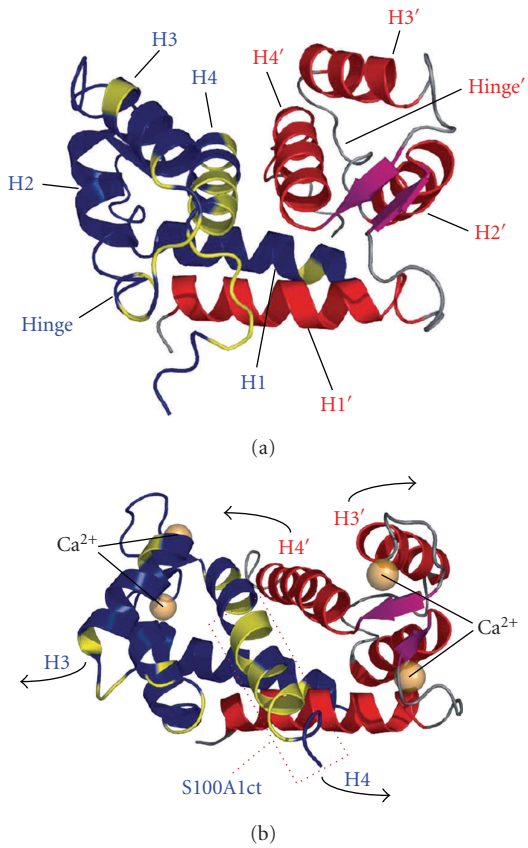

Figure 2: The three-dimensional structure of S100A1 as determined by NMR spectroscopy. (a) S100A1 in the apo state: S100A1 is composed of two identical subunits connected by a linker region (hinge region). Dimerization occurs in an antiparallel manner. (b) S100A1 in the Ca^{2+} bound state: Ca^{2+} binding to both the N- and C-terminal motif results in an altered orientation of H3/4 and the hinge region uncovering hydrophobic residues for the interaction with target molecules. S100A1 residues 75–94 are indicated by the red box. Reproduced with modifications from Wright et al. [7].

In contrast, heterozygous and homozygous S100A1 knockout (SKO) mice showed unaltered in vivo baseline cardiac function and heart rate, but displayed deficiencies in their contractile function in response to β-AR stimulation and enhanced transsarcolemmal Ca^{2+} influx [36].

This is interesting to note because heterozygous SKO hearts, with only 50% of S100A1 protein levels found in wild-type hearts, exhibit the same acute defects than homozygous SKO hearts with complete lack of S100A1 expression. Thus >50% of normal left-ventricular S100A1 protein levels are apparently required for cardiac adaptation to acute hemodynamic stress. Of note, loss of β-AR-dependent inotropy in SKO mice occurs despite regular β-AR signaling ranging from unaltered β-AR density to proper PKA-dependent target phosphorylation, thereby indicating that β-AR-mediated positive inotropy essentially relies on normal S100A1 protein levels in the heart.

Additional exploration of isolated S100A1 knock-out cardiomyocytes revealed blunted Ca^{2+} transients in response to sympathetic stimulation and increased extracellular $[Ca^{2+}]$ [31]. This is mainly mediated by a decreased Ca^{2+} release (and reduced SR Ca^{2+} load). Similar results were obtained in neonatal ventricular cardiomyocytes, with a S100A1 protein knockdown to 20–30% of control protein levels [22].

Thus defective mobilization of Ca^{2+} from intracellular stores might provide a reasonable mechanism for the impaired inotropic reserve in S100A1-deficient and -depleted cardiomyocytes. In line with this, murine S100A1 knock out ventricular cardiomyocytes have enhanced l-type Ca^{2+} current $[I_{Ca,L}]$ (Most P, unpublished data), which indicates that desensitized Ca^{2+} induced Ca^{2+} release (CICR) under basal conditions might be compensated by increased $[I_{Ca,L}]$.

4.2. Molecular Targets: Sarcoplasmic Reticulum in Cardiac Myocytes. Studies focused on mechanisms underlying S100A1 inotropic actions provided evidence that S100A1 targets SR but not transsarcolemmal Ca^{2+} fluxes in ventricular cardiomyocytes. Several studies identified the impact of S100A1 on intracellular Ca^{2+} homeostasis and consistently found that S100A1 increases systolic and diastolic performance through an enhanced Ca^{2+} induced Ca^{2+} release and augmented SR Ca^{2+} reuptake [17–19, 21, 27, 31]. At the subcellular level, endogenous S100A1 displays a striated like pattern in ventricular cardiac myocytes and is found at the junctional and longitudinal sarcoplasmic reticulum. Accordingly, S100A1 was found to colocalize and interact with SERCA2 and RyR2 in a Ca^{2+}-dependent manner in human, mouse and rat myocardium.

In line with Ca^{2+}-dependent conformational changes, binding studies identified the S100A1 COOH terminus (S100A1ct) as the critical structure for the interaction with the SERCA-PLB complex [18]. Functional analysis in isolated SR vesicles and intact cardiomyocytes showed that S100A1 apparently stimulated SERCA2 activity resulting in increased SR Ca^{2+} reuptake and enhanced SR Ca^{2+} load. In isolated SR vesicles a similar increase in SERCA activity was obtained with S100A1ct consisting of the c-terminal amino acid residues 75–94, which indicates the crucial role of helix IV for the Ca^{2+} dependent functions of S100A1[18].

The underlying molecular mechanism has not been explored yet, but it is important to note that neither S100A1 nor S100A1ct affects PLB phosphorylation at serine 16 or threonine 17 sites, suggesting that neither PKA nor CaMKII are involved in S100A1-mediated SERCA2 regulation. This might also indicate that S100A1 does not limit access of these kinases to their specific phosphorylation sites [17].

S100A1 binding to the RyR also seems to be Ca^{2+}-dependent since mapping studies also identified S100A1ct as the critical S100A1 epitope for its RyR2 interaction [27]. Recent studies found that S100A1 can bind to the calmodulin (CaM) binding domain in the cytoplasmic portion of skeletal ryanodine receptor isoform (RyR1), which is highly conserved between RyR1 and RyR2 [37, 38]. S100A1 modulates both diastolic and systolic RyR2 functions and thereby alters frequency and characteristics of elementary SR Ca^{2+} events

(Ca^{2+} sparks) and Ca^{2+} induced SR Ca^{2+} release, respectively. At nanomolar free Ca^{2+} concentrations, S100A1 decreased ^{3}H-ryanodine binding to the SR Ca^{2+} release channel, suggesting an inhibitory effect on RyR2 opening under diastolic conditions [18, 20]. In line with this, S100A1 interaction with RyR2 even at diastolic Ca^{2+} concentration [150 nM] decreased Ca^{2+} spark frequency and diastolic SR Ca^{2+} leak in quiescent rabbit cardiac myocytes and cardiac SR vesicle preparations, which indicates that S100A1 can improve RyR2 closure during cardiomyocyte relaxation [27]. In contrast, at micromolar systolic Ca^{2+} concentrations S100A1 provoked increased ^{3}H-ryanodine binding in isolated SR vesicles [18]. Accordingly, a recent study confirmed that S100A1 increased fractional Ca^{2+} release of the SR in voltage-clamped rabbit cardiomyocytes, suggesting that S100A1 can improve excitation-contraction coupling gain in cardiac myocytes under systolic conditions [20]. Taken together, these data support the hypothesis that S100A1 promotes RyR2 closure during diastole but can augment systolic RyR2. The differential effects predict more than only one binding site at the RyR2 and this notion is further supported by recent mapping studies. At least three different S100A1 binding domains have been identified with different binding affinities within the cytoplasmic portion of the RyR [39]. Further functional analyses confirmed that of S100A1 actions on diastolic RyR2 function neither changed FKBP12.6 not sorcin stoichiometry with the RyR2 channel [27]. Therefore it is attractive to hypothesize that both inhibitory and stimulatory bindings sites for S100A1 might coexist at the RyR2, which eventually account for physiological normal diastolic and systolic performance of the SR Ca^{2+} release channel. Moreover, given the redox sensitivity of the RyR, NO-dependent posttranslational modifications of S100A1 might contribute to the actions on the SR release channel, that is, through transfers of NO moieties. In summary, the precise mechanism underlying S100A1-mediated regulation of RyR2 remains to be determined, but evidence points toward a biphasic modulation of RyR2 activity.

Interestingly, it has been shown that S100A1 neither altered L-type Ca^{2+} current nor sodium-calcium exchanger (NCX) reverse or forward mode in adult ventricular cardiomyocytes [27] and similar results were found for intracellular S100A1 in neonatal ventricular cardiac myocytes [21]. These results further support the notion that enhanced cardiomyocyte S100A1 proteins do not evoke cardiac hypertrophy, because the S100A1 Ca^{2+} sensor does not seem to enhance subsarcolemmal Ca^{2+} fluxes participating in Ca^{2+} dependent hypertrophic cardiac growth [19, 31].

4.2.1. Molecular Targets: Sarcomere. Inotropic actions of S100A1 are apparently not restricted to the SR, because S100A1 has been shown to regulate both myofilament Ca^{2+} sensitivity and diastolic compliance. S100A1 has been detected at different sites within the cardiac sarcomere, suggesting a possible role in myofilament function [23, 24, 40–42]. S100A1 has been detected at the Z-line, at the periphery of the M-lines as well as within I- and A-bands. In the I-band, Ca^{2+}-dependent interaction with the PEVK subdomain of titin has been shown to result in improved sarcomeric compliance [23, 24]. It remains to be established if the binding partner in the A-band is also titin or another thick-filament protein. Granzier and colleagues hypothesized if S100A1 does bind titin in the A-band, the location of the binding sites suggests interaction with titin's super-repeat domains.

Based on their results, Granzier and colleagues hypothesized that S100A1 inhibition of titin-actin interaction might result in reduced precontractile titin-based passive tension. In addition, it has been shown that S100A1 can reduce myofilament Ca^{2+} sensitivity without affecting maximal force development and PKA- and PKC-dependent troponin I phosphorylation. As a result, S100A1 might facilitate diastolic Ca^{2+} dissociation from myofilaments thereby improving cardiac relaxation by an additional, SR-independent mechanism. At present, it is unknown whether S100A1 has additional sarcomeric targets warranting further studies to determine the functional role of S100A1 location at Z-disc adjacent protein structures.

4.2.2. Molecular Targets: Additional Targets-Mitochondria. Böerries et al. provided first evidence that S100A1 is found in cardiac mitochondria by immunofluorescence and immunoelectron microscopy. In the mitochondria S100A1 interacts with the F1-ATPase in a Ca dependent manner [22]. This resulted in enhanced activity of the ATP generation. The same study identified the adenine nucleotide translocator (ANT) as a mitochondrial S100A1 target. This suggests that interaction of S100A1 with ANT might influence the ADP and ATP exchange between the mitochondria and the cytoplasm. This might also be involved in the regulation of cardiac apoptosis, as another study yielded antiapoptotic effects of S100A1 in isolated neonatal rat ventricular myocytes [28]. In line with this, decreased S100A1 protein levels resulted in a reciprocal energetic phenotype with decreased ATP-levels and enhanced cardiomyocyte apoptosis. These findings support a crucial role for S100A1 in cardiac energy homeostasis [22]. Additional studies are underway to clarify the impact of S100A1 on the interplay between the SR and mitochondria.

4.3. Cardiomyocyte Ca^{2+} Handling and Contractile Performance. S100A1 plays an important role in cardiac myocytes Ca^{2+} cycling and contractile performance. Based on the molecular targets, numerous studies have shown that the overall effect of increased S100A1 protein levels can increase intracellular Ca^{2+} cycling, SR Ca^{2+} load, and contractile performance in field-stimulated mice, rat, and rabbit ventricular myocytes. These changes in cardiomyocyte performance most likely reflect altered function of S100A1 molecular targets as discussed above. Given the fact that S100A1 enhances both SERCA2 activity and diastolic RyR2 diastole, combined actions most likely contribute to enhanced SR Ca^{2+} load and accelerated cardiomyocyte relaxation. In addition, improved myofilament Ca^{2+} dissociation in concert with reduced presystolic passive tension might contribute to improved diastolic function. In systole, increased SR Ca^{2+}

load, in concert with enhanced systolic RyR2 opening eventually represents a powerful mechanism by which S100A1 can augment systolic Ca^{2+} transients and cardiac contractile performance (Figure 3).

In summary, several important conclusions and hypotheses can be drawn from these results: first S100A1 is a molecular inotrope in cardiomyocytes exerting a PKA-independent enhancement of cardiac Ca^{2+} cycling thereby acting beyond and independently of cAMP-dependent signaling. Secondly, S100A1 specifically targets SR Ca^{2+} fluxes, improving both systolic SR Ca^{2+} release capability (enhances systolic RyR2 activity) and diastolic SR Ca^{2+} storage capability (decreases diastolic RyR2 open probability and enhances SERCA2a activity. The synchronous enhancement of SERCA2a activity and decreased RyR2 activity in diastole is a hitherto unique and potentially synergistic molecular mechanism which amplifies its inotropic actions but might help to prevent Ca^{2+}-triggered arrhythmias; finally, S100A1 modulation of cardiac titin function might adapt cardiac passive tension and sarcomeric Ca^{2+} sensitivity to improved Ca^{2+} cycling, thereby facilitating diastolic cardiac performance. Finally, S100A1 mitochondrial actions might reflect a mechanism to couple Ca^{2+}-dependent energetic demand with mitochondrial energy production. Figure 3 provides a scheme to illustrate the molecular mechanism of S100A1 in a normal cardiomyocyte.

4.4. Skeletal Muscle Ca^{2+} Handling. Skeletal muscle expresses S100A1 at lower levels than cardiac muscle [30]. However, numerous studies have demonstrated an important role of S100A1 in skeletal muscle EC-coupling [30, 37, 38, 43–45]. S100A1 binds to RyR1 and potentiates its open probability, which might be regulated by three identified S100A1 binding sites at the RyR1 [39]. Interestingly, one of the binding site overlaps with a Ca^{2+}-dependent CaM binding domain. In saponin skinned muscle fibers, addition of recombinant S100A1 protein enhanced SR Ca^{2+} release and isometric force development in a dose-dependent manner both in fast- and slow-twitch skeletal muscle fibers [30] and maximal effects were seen at nanomolar S100A1 protein concentrations. However, S100A1 neither activated RyR1 in its closed state nor initiated SR Ca^{2+} release at diastolic Ca^{2+} concentrations in the presence of physiologic Mg^{2+} concentrations. In contrast to cardiac myocytes, S100A1 neither enhanced SR Ca^{2+} load nor altered Ca^{2+} sensitivity of skeletal muscle myofilaments indicating that S100A1 action in skeletal muscle might be limited to SR Ca^{2+} release sites and eventually mitochondria [30].

In S100A1 deficient skeletal muscle fibers Wright et al. most recently found that voltage-induced SR Ca^{2+} release is decreased resulting in diminished global Ca^{2+} transients and contractile force development [37]. The same study revealed that viral-based S100A1 gene delivery can rescue the phenotype providing ultimate evidence for the hypothesis that S100A1 plays a significant physiological role in skeletal muscle EC-coupling. At a molecular level, the authors further showed that S100A1 and CaM can compete for the same binding site at the RyR1 in a Ca^{2+}-dependent manner and identified RyR1 residues 3416–3427 as the putative common binding domain [38].

Interestingly, this interaction involves the hydrophobic pocket of S100A1, which is exposed in its Ca^{2+} activated state. At this point it is interesting to note that the S100A1/RyR1 binding domain is involved in the intersubunit interactions of the RyR1 as well as close in space to distal parts of the channel, so that the authors developed a model where S100A1 proteins might be involved in linking one or two subunits of the RyR tetramer. Recently another interesting study by the same group showed that decreased SR Ca^{2+} release in S100A1 KO fibres is responsible for the suppression of the temporally delayed component of intramembrane charge movement, Qgamma, which might operate as an indicator of optimized local Ca^{2+} release at the triad junction in skeletal muscle [44, 45]. In regard of the previously discussed importance of S100A1 in cardiac muscle, it is tempting to speculate that S100A1 plays a key role in skeletal muscle physiology.

Taken together, these data indicate that S100A1 plays a significant role in skeletal muscle EC coupling through modulation of RyR1 function, but not through modulation of SR Ca^{2+} load. Further studies are necessary to determine the impact of S100A1 in skeletal muscle disease to determine the pathophysiological role a therapeutic potential of S100A1 in skeletal muscle disorders like congential myopathies.

5. S100A1 in Diseased Myocardium

The discovery of altered S100A1 protein abundance in failing human myocardium finally raised the question for a therapeutic potential of S100A1 since progressively diminished S100A1 mRNA and protein levels characterize failing human myocardium [46]. A recent proof of concept study employing both cardiac S100A1 transgenic and knock-out mouse models has provided evidence that diminished S100A1 protein levels critically contribute to progressive contractile dysfunction in postischemic heart failure (HF) and death [31]. The same study indicated that preserved cardiomyocyte S100A1 protein levels could actually prevent development of postischemic HF and cardiac death. In line with S100A1 molecular actions in isolated cardiomyocytes, remote myocardium from infarcted S100A1-overexpressing hearts showed preserved SR Ca^{2+} handling while S100A1 deficiency resulted in abnormal SR Ca^{2+} content and fluxes. In addition, S100A1 knock-out mice subjected to transaortic constriction showed accelerated deterioration of cardiac performance and transition to heart failure [36]. Therefore, normal S100A1 cardiac expression levels appear to be essential to cope with increased workload due to ischemic loss of myocardium or chronically elevated afterload.

Abnormal cardiomyocyte S100A1 protein levels have further been recapitulated in vitro by chronic stimulation with different hypertrophic stimuli like phenylephrine and endothelin-1 [31]. In light of the low abundance of S100A1 in neonatal and developing hearts, downregulation of S100A1 in the course of cardiac hypertrophy could therefore be considered as a part of fetal gene reprogramming.

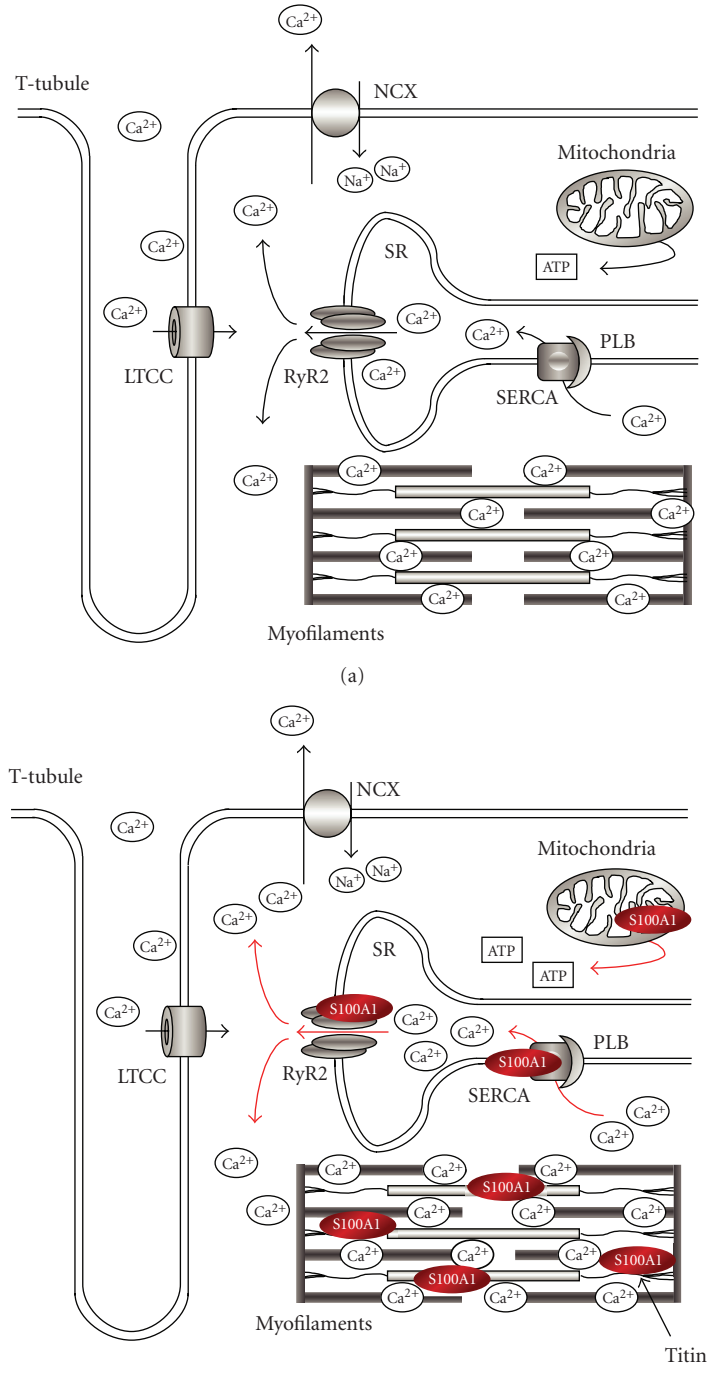

Figure 3: Proposed model for S100A1 inotropic actions in cardiomyocytes. (a) During excitation-contraction coupling, the action potential-dependent opening of the L-type Ca^{2+} channel (LTCC) results in a transsarcolemmal Ca^{2+} entry which triggers sarcoplasmic reticulum (SR) Ca^{2+} release via Ryanodine Receptor 2 (RyR2) that in turn activates myofilament cross-bridge cycling and mechanical contraction. During diastole, the SR Ca^{2+} reuptake is conducted by the SR Ca^{2+} ATPase (SERCA), whereas the sodium-calcium exchanger (NCX) extrudes Ca^{2+} from the cardiomyocyte to keep steady-state conditions. (b) S100A1 interacts with both RyR2 and the SERCA-Phospholamban (PLB)-complex and is present at myofilaments and mitochondria. Increased S100A1 protein levels result in an enhanced systolic SR Ca^{2+} release via RyR2 without influencing LTCC activity. Augmented SR Ca^{2+} release is balanced by an intensified SERCA activity leading to an improved Ca^{2+} cycling and a raised force generation. Additionally, the S100A1/F1-ATPase interference in mitochondria is associated with an enhanced generation of cytoplasmic ATP in cardiomyocytes. Moreover, S100A1 inhibits the actin-titin interactions in the sarcomere, resulting in a reduced precontractile passive tension [24].

The binding of S100A1 to the sarcomere could contribute to an altered diastolic function in diseased myocardium. Reduced binding of S100A1 to titin in this context could theoretically contribute to an increased precontractile titin-based passive tension. Interestingly, the myofilament relaxation seems to be dependent on the intracellular ATP-balance [47]. Because S100A1 deficiency reduced intracellular ATP-levels in cardiac myocytes (see above), it is tempting to speculate that lower ATP-levels due to S100A1 deficiency contribute to impaired diastolic function in cardiac disease. Moreover, it has been shown that S100A1 affects microtubules stability in the presence of Ca^{2+} [48]. Given the fact that recent work has shown that microtubules increase viscosity in cardiac hypertrophy and heart failure [49], further studies are needed to clarify the impact of altered S100A1 protein expression on diastolic function in cardiac diseases.

6. Therapeutic Implications and Clinical Perspectives

Owing to the pathophysiological relevance of altered S100A1 expression in cardiac disease and therapeutics effectiveness in clinically relevant experimental acute and chronic HF animal models, S100A1 might be considered as a future prototype of a novel class of Ca^{2+}-dependent inotropes. Intracoronary S100A1 adenoviral and adeno-associated viral gene delivery restored abnormal S100A1 expression in failing hearts thereby improving cardiac performance and reversing cardiac hypertrophy in vivo [15, 18, 32, 33]. So far, both RyR2 and SERCA have been considered as major therapeutic S100A1 targets in failing myocardium given normalization of dysfunctional Ca^{2+} cycling via restored SR Ca^{2+} content, improved systolic SR Ca^{2+} release, as well as decreased diastolic SR Ca^{2+} leakage. Because S100A1 augments both SR Ca^{2+} load and fractional SR Ca^{2+} release, it is tempting to speculate that S100A1 promotes inotropic SR Ca^{2+} handling but potentially limits Ca^{2+} triggered arrhythmias by stabilizing the RyR2 during diastole. This notion is based on the observation that S100A1 gene delivery to normal and failing cardiomyocytes can prevent β-AR triggered proarrhythmic SR Ca^{2+} leak and diastolic Ca^{2+} waves. Accordingly, S100A1 knock-out mice display enhanced proarrhythmic susceptibility in response to sympathetic stimulation [50] and prevention of this effect might actually contribute to improved postMI survival in S100A1 transgenic mice [51]. These findings might further substantiate the clinical relevance of S100A1-mediated regulation of SR Ca^{2+} handling through balanced modulation both of RyR2 and SERCA2. Moreover, S100A1 gene delivery in diseased myocardium exerts robust antihypertrophic effects in vivo and prevents progressive left ventrical chamber dilatation, potentially reflecting abrogated wall stress and interrupted sympathetic overdrive. Finally, S100A1 gene therapy also restored defective energy and sodium homeostasis which are closely linked to Ca^{2+} handling abnormalities [18] clearly warranting father investigation of the underlying molecular mechanisms.

From a clinical point of view, it is important to point out that viral-based S100A1 gene therapy has proven its therapeutic effectiveness both in an experimental postischemic large-animal HF model [52] and human failing ventricular cardiomyocytes (Most P, unpublished observation). Retroinfusion-facilitated delivery of AAV9-S100A1 gene via the anterior coronary vein 2 weeks postMI restored left ventricular performance and prevented transition to contractile failure as seen in control groups. In line with sustained improvement of cardiac performance, S100A1 gene therapy reversed cardiac remodeling and sympathetic overdrive [52]. Moreover, another proof of concept study provided evidence that S100A1 gene therapy normalized abnormal contractility and SR Ca^{2+} handling in failing human cardiomyocytes and restored a positive force-frequency response in these cells (Most P, unpublished observations). These translational studies might actually pave the way for novel S100A1-based clinical therapies including S100A1 gene therapy of human HF. However before S100A1 HF gene therapy can become a clinical reality, careful evaluation of its safety profile as well as effects on cardiac energetics, arrhythmias and compatibility with established pharmacological therapies needs to be conducted in large-animal models. Importantly, a recent study actually provided first evidence that S100A1 gene therapy neither interferes with sympathetic stimulation nor β-blocker treatment [32].

7. Summary

To improve human health, scientific discoveries must be translated into practical applications. Such discoveries typically begin with clinical observations further requiring basic research at "the bench" to uncover molecular mechanisms and proof of pathophysiological relevance before progressing to the clinical level, or the patient's "bedside." The clinical discovery of altered S100A1 protein abundance in failing human myocardium [46] ignited a challenging search for its pathophysiological relevance and potential therapeutic impact. Now, almost a decade later, ample evidence indicates that S100A1 functions as a Ca^{2+}-dependent molecular inotrope in cardiomyocytes with unique therapeutic properties. S100A1 targets several key regulators of SR, myofilament, and mitochondrial function thereby enhancing cardiomyocyte performance. In addition, several lines of evidence indicates that S100A1 plays a significant role in skeletal muscle function but its pathophysiological relevance and therapeutic potential in skeletal muscle disorders have not been fully explored yet. Dysregulation of cardiomyocyte S100A1 protein expression, however, accelerates development of cardiac hypertrophy, progression towards HF and cardiac death. Translational studies provided evidence for long-term rescue of cardiac performance and reversed remodeling in several small and large HF animal models. Importantly, S100A1 therapeutic actions extend to human failing myocardium showing restored contractile performance based on improved SR Ca^{2+} handling in S100A1-treated human failing cardiomyocytes [17]. Due to these findings, S100A1-based HF therapies further progress to

the clinical level moving forward towards first clinical safety trials. One could therefore speculate that S100A1 gene therapy might be applicable both to acute and chronic cardiac dysfunction potentially benefiting patients with acute cardiac decompensation as chronic HF. In theory, S100A1 gene therapy for HF may offer a promising and novel mode of action that will not only promote beneficial cardiomyocyte EC Ca^{2+} handling but also potentially limit arrhythmias through stabilization of RyR and decrease in diastolic Ca^{2+} leak. Moreover, the impact of S100A1 on the sarcomere and the mitochondria function completes the unique therapeutic potential of S100A1.

However, development of S100A1-based therapeutic will presumably not replace currently established drug regimens but rather complement and add on to these therapeutic strategies. Nevertheless, S100A1 might facilitate a clinical revival of inotropic therapeutic interventions by targeting defective Ca^{2+} cycling and providing future cardiologists with a novel weapon to combat heart failure.

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