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Differential effects of S100 proteins A2 and A6 on cardiac Ca²⁺ cycling and contractile performance

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Abstract

Defective intracellular calcium (Ca²⁺) handling is implicated in the pathogenesis of heart failure. Novel approaches targeting both cardiac Ca²⁺ release and reuptake processes, such as S100A1, have the potential to rescue the function of failing cardiac myocytes. Here, we show that two members of the S100 Ca²⁺ binding protein family, S100A2 and S100A6 that share high sequence homology, differentially influence cardiac Ca²⁺ handling and contractility. Cardiac gene expression of S100A2 significantly enhanced both contractile and relaxation performance of rodent and canine cardiac myocytes, mimicking the functional effects of its cardiac homologue, S100A1. To interrogate mechanism, Ca²⁺ spark frequency, a measure of the gating of the ryanodine receptor Ca²⁺ release channel, was found to be significantly increased by S100A2. Therapeutic testing showed that S100A2 rescued the contractile defects of failing cardiac myocytes. In contrast, cardiac expression of S100A6 had no significant effects on contractility or Ca²⁺ handling. These data reveal novel differential effects of S100 proteins on cardiac myocyte performance that may be useful in application to diseased cardiac muscle.

Keywords

S100 proteins; Cardiac calcium handling; Excitation-contraction coupling; Heart failure; Gene therapy

1. Introduction

Heart failure (HF) is a major health problem and a leading contributor to the increasing financial burden for patients and the health care system [1]. Despite numerous efforts in developing novel treatments, current therapeutic approaches are primarily palliative in

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Conflict of interest statement

None.

nature with little success in reversing or preventing the disease [2]. In HF, various intracellular or extracellular stresses contribute to the progressive decline of cardiac output through persistently damaging myocardial structure and function [3–5]. One important feature of HF is the dysregulation of intracellular calcium (Ca^{2+}) handling as seen in altered Ca^{2+} release, slow or incomplete Ca^{2+} reuptake and elevated resting diastolic Ca^{2+} levels [2, 6–9]. Therefore, targeting intracellular Ca^{2+} cycling has been the focus of many studies, in which genetic or pharmacological manipulations of endogenous Ca^{2+} handling proteins, such as the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a), the ryanodine receptor Ca^{2+} release channel (RyR) and the S100A1 Ca^{2+} binding protein, have been shown to benefit heart performance [2, 10–12].

There are a number of potential Ca^{2+} handling proteins and pathways for targeting in HF including the S100 protein super family. The S100 proteins constitute the single largest subgroup within the EF-hand Ca^{2+} -binding motif super family [13]. S100 proteins have been shown to modulate a great variety of physiological and pathological processes, such as enzyme activities, Ca^{2+} transportation, energy metabolism, motility, secretion, gene transcription, apoptosis and tumorigenesis [14–17]. Due to their moderate Ca^{2+} binding affinity ($K_d > 50 \mu\text{M}$) [18], S100 proteins are generally regarded as Ca^{2+} sensors rather than Ca^{2+} buffers. Ca^{2+} -induced conformational changes allow S100 proteins to interact with cognate target proteins to modulate their function [19]. In cardiac muscle, S100A1 is the predominant S100 family member, while S100A4, S100A6, and S100B are also expressed with much less abundance [20]. The physiological role of S100A1 in the heart has been extensively studied and shown to directly enhance cardiac Ca^{2+} cycling, interact with the myofilaments, and regulate mitochondrial metabolism [21]. In particular, S100A1 facilitates both Ca^{2+} release and Ca^{2+} reuptake and thereby promotes myocyte contractility [19]. These effects are mainly derived from the direct or indirect interactions between S100A1 and Ca^{2+} handling proteins, including RyR and SERCA2a, and subsequent stimulation of their activity [21, 22]. Evidence has accumulated to show that S100A1 may have therapeutic potential in heart failure [23, 24].

Other than S100A1, very little is known about other S100 proteins that may enhance heart function. In noting the marked sequence homology among S100A proteins (Fig. 1A), we hypothesized that close structural relatives of S100A1, notably S100A2 and S100A6 will have unique physiological functions in cardiac muscle and show benefit to myocytes from failing hearts. S100A2 is broadly expressed in many tissues including lung, kidney and skeletal muscles [25] and is detectable in human and bovine heart but not in rodent heart [26]. S100A6 is also widely expressed in many tissues including the human heart [27] and is predominantly a cytoplasmic protein that binds with many protein partners [28]. The absence of endogenous S100A2 and S100A6 proteins in the normal rodent heart offers a template to test de novo the ectopic expression of these proteins to dissect out structure-function. We used gene transfer to express S100 family members S100A2, and separately S100A6, for comparison to S100A1 in cardiac myocytes. Interestingly, S100A2, but not S100A6, significantly increased myocyte contractility and enhanced relaxation performance. The positive inotropic and lusitropic effects of S100A2 were supported by its effects on Ca^{2+} release channels and affecting faster Ca^{2+} decay. Therapeutic testing showed that S100A2 gene transfer can rescue both systolic and diastolic dysfunction of the failing

myocytes. These results uncover differential effects of S100 proteins on cardiac myocyte function and point to S100A2 as a new candidate in heart failure therapy.

2. Methods

2.1. Adenovirus production

Full length human S100A1, S100A2 or S100A6 cDNAs were purchased from Invitrogen and first cloned into a pDC316 shuttle vector (Invitrogen). Recombinant adenovirus containing S100A1, S100A2 or S100A6 was generated by using AdMax™ (from Microbix) system as described previously [29]. Gene expression of each vector was controlled by the cytomegalovirus (CMV) promoter and polyadenylation signal provided by simian virus 40. High-titer (10^{11} – 10^{12} plaque forming unit, PFU) and plaque-purified adenoviral stocks were produced and purified and viral aliquots were stored at -80°C .

2.2. Adult cardiac myocyte isolation, culture and gene transfer

The procedures used in this study were in agreement with the guidelines of the Internal Review Board of Institutional Animal Care and Use Committee of the University of Minnesota. Veterinary care was provided by the Research Animal Resources at the University of Minnesota. Adult cardiac myocytes were enzymatically isolated from the left ventricle of rats as described previously [29, 30]. Briefly, Sprague-Dawley rats (~200g) were anesthetized by 162.5 mg/Kg Nembutal with 1500 U/Kg heparin (i.p.). Heart was quickly removed, mounted on a modified Langendorff apparatus, and perfused with oxygenated KHB solution (in mM: NaCl, 118; KCl, 4.8; HEPES, 25; K_2HPO_4 , 1.25; MgSO_4 , 1.25; Glucose, 11; pH 7.4) with 1 mM CaCl_2 for 5 min. The heart was further perfused with oxygenated KHB solution without CaCl_2 for 5 min and perfused with type II collagenase (0.5mg/mL) and hyaluronidase (0.2mg/mL) for 15–20 min. Left ventricle was cut into small pieces and individual cardiac myocytes were collected through gentle agitation of the tissue. Single cardiac myocytes were plated on laminin-coated glass coverslips with a density of 2×10^4 myocytes/coverslip. Myocytes were transduced with AdS100A1, AdS100A2 or AdS100A6 at a multiplicity of infection (MOI) of 500 and cultured in M199 media (Sigma) supplemented with 10 mM glutathione, 26.2 mM sodium bicarbonate, 0.02% bovine serum albumin and 50 U/ml penicillin-streptomycin for up to 4 days [31]. Isolation of adult canine cardiac myocyte followed previously reported methods [32].

2.3. Determination of gene expression

Detection of protein expression after adenovirus mediated gene transfer was performed by Western Blot and immunofluorescent assays. One to three days after gene transfer, myocytes were harvested in Laemmli sample buffer, and protein samples were loaded on SDS-PAGE gel. Separated proteins were transferred to nitrocellulose membrane and probed with monoclonal antibodies specific for S100A1 (1:500, Acris Antibodies, Inc.), S100A2 or S100A6 (1:200–1000, Sigma), calsequestrin (CSQ, 1:1000, ABR), phospholamban (PLN, 1:1000, Abcam), SERCA2a (1:500, Sigma), and $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX, 1:500, Chemicon). Actin, using anti-actin antibody (1:2500, Sigma), was used for loading control. Secondary antibodies were conjugated to IRDye 800 (Rockland) or Alexa Fluor 680

(Molecular Probes) and signals were visualized and quantified using the Odyssey system (Licor). Protein bands were quantified using commercially available software (Quantiscan). For immunofluorescence assays, cells were fixed with 3% paraformaldehyde and incubated first with primary antibodies for S100 protein and then with fluorescent probe conjugated secondary antibodies (anti-mouse IgG-FITC or anti-rabbit IgG-Texas red, 1:1000, Molecular Probes). Fluorescence images were taken by a fluorescent microscope (Nikon) or confocal microscope (Olympus).

2.4. Measurement of single cardiac myocyte contractility

Contractile function was measured three days after *in vitro* gene transfer by the Myocyte Calcium and Contractility Measurement System (IonOptix) as previously described [33]. In brief, a coverslip containing cardiac myocytes was transferred to form a stimulation chamber on the stage of a microscope (Nikon, Eclipse TE2000). The chamber was filled with M199 medium and myocytes were electrically stimulated (60 V, 0.2 Hz) by MyoPacer (IonOptix). Myocytes were viewed under a 40× objective (N.A. 1.3) and images were collected (240Hz) by a CCD camera (MyoCam, IonOptix). Sarcomere length (SL) shortening was collected by real-time fast Fourier transform of the myocytes SL video signal using commercially available data acquisition software (IonOptix). Experiments were performed at room temperature or 37°C as indicated. Only myocytes with a resting SL >1.75 μm were used for the study. The myocyte transient analysis software (IonOptix) was used to determine baseline SL, peak SL shortening, percentage SL shortening, time to maximal peak shortening (Tp) and time from peak to 25, 50, 75 and 90% relaxation (T25%, T50%, T75% and T90%, respectively). Cardiac stress testing was by ramp increase in field stimulation frequency from 0.2 to 2 Hz.

2.5. Measurement of myocyte Ca²⁺ handling

For Ca²⁺ transient measurement, myocytes were loaded with fura-2/AM (a ratiometric Ca²⁺ indicator, 2 μM, Molecular Probes) for 10 min at room temperature following deesterification in M199 for 20 min [34]. Fura-2 fluorescence was measured using a spectrophotometer (Stepper Switch, IonOptix). Initially, fura-2 was excited at 360nm (the isosbestic point independent of Ca²⁺) and then continuously at 380nm (Ca²⁺ dependent fluorescence). Emission was collected at >510 nm by a photomultiplier tube. Ratiometric (360/380) data were collected from single cardiac myocyte with field stimulation (0.2 Hz) and Ca²⁺ transient parameters were analyzed using commercial software (IonOptix). To measure caffeine-induced sarcoplasmic reticulum (SR) Ca²⁺ release, fura-2 loaded myocytes were electrically stimulated (1 Hz) for 1 min followed by local and transient perfusion of caffeine (20 mM) using a rapid capillary tube perfusion system. In a subset of experiments, Na⁺ and Ca²⁺ were removed from the incubating solution to suppress NCX and caffeine was perfused continuously. Visualization of spontaneous Ca²⁺ sparks used fluo-4 Ca²⁺ indicator and confocal laser-scanning microscope (LSM510, Carl Zeiss), following methods described previously [35]. All the functional experiments were performed at 1.8 mM CaCl₂ in M199 medium.

2.6. Heart failure model

The left anterior descending artery (LAD) ligation model was used to produce heart failure in rats as previously described [36]. Briefly, after induction, endotracheal intubation with a 16-gauge angiocatheter was performed. Rats were supported by a small animal ventilator (Kent Scientific) and maintained with isoflurane (Hospira) during surgery. A left thoracotomy was performed to expose the anterior surface of the heart. The proximal LAD was identified and a 7-0 prolene suture placed around the artery and surrounding myocardium. The landmarks used for the ligation placement included the line (1–2 mm distal) between the left border of the pulmonary conus and the right border of the left atrial appendage. Ischemia was confirmed with ST-segment elevation on electrocardiogram. The pneumothorax was evacuated using saline. The ribs were reapproximated and incision closed in two layers. Postoperative care was continued using the MouseOx blood oxygen monitoring (Starr Life Sciences) until extubation and full recovery from anesthesia. Echocardiography was performed using an HP Sonos 5500 ultrasound machine to verify myocardial infarction and to measure cardiac function 2–4 weeks after the surgery.

2.7. Statistics

Data were expressed as Mean $\pm$ SEM. One-way ANOVA and unpaired *t* test were used when appropriate to determine statistical significance among groups. A *P* value of less than 0.05 was deemed significant.

3. Results

3.1. Functional screening of S100 proteins

Analysis of the primary amino acid sequence of S100A1, S100A2 and S100A6 revealed high homology between these S100 family members: 50% identical in amino acids and 66% overall homology (Fig. 1A). To determine whether S100A2 and S100A6 have direct functional effects on cardiac contractility and Ca^{2+} cycling, we employed adenovirus-mediated gene expression of human S100A2 or S100A6 in adult rat cardiac myocytes (AdS100A2 and AdS100A6, respectively, Fig. 1B). Robust expression of S100A2 or S100A6 was detected 2–3 days after gene transfer by immunostaining and Western blot analysis (Fig. 1C–D) indicating high gene transfer efficiency. Interestingly, S100A2 expression was detected in the cytosol exhibiting striated pattern co-aligning with the actin containing thin myofilaments (Fig. 1C enlarged view), while S100A6 distribution in the cytosol is more homogeneous. The ectopic expression of S100A2 did not significantly affect the level of endogenous S100A1 in rat myocytes (Fig. S1).

To test whether expression of S100A2 or S100A6 could affect contractility, cardiac myocytes were electrically stimulated at 0.2 Hz and sarcomere length (SL) shortening during each twitch was monitored. S100A2 significantly increased myocyte contraction amplitude (SL shortening from $6.97 \pm 0.80\%$ in control myocytes to $10.79 \pm 0.94\%$ in AdS100A2 treated cells, $P < 0.05$, $n = 25$ –40, Fig. 2A, C), decreased relaxation duration (time from peak to 50% relaxation, T50%, from 156.3 ± 13.5 ms in control cells to 107.1 ± 6.5 ms in AdS100A2 myocytes, T75% from 333.2 ± 34.3 ms to 222.6 ± 14.4 ms, and T90% from 790 ± 78.3 ms to 510.4 ± 41.8 ms, $P < 0.05$, Fig. 2B, D), and increased contraction and

relaxation velocity (Fig. 2E–F). In comparison, S100A6 had no significant effect on myocyte contraction amplitude or relaxation (Fig. 2). Enhanced systolic and diastolic function in cardiac myocytes after S100A2 expression was comparable to that observed with the overexpression of S100A1 (Fig. S2), and in our hands S100A1 had similar effects to those reported previously for this S100 family member [37].

3.2. S100A2 modulates Ca^{2+} transients in cardiac myocytes

To further elucidate the mechanism underlying S100A2's effects on myocyte contraction and relaxation performance, we monitored intracellular Ca^{2+} transients using the ratio metric fluorescent Ca^{2+} indicator, fura-2. As shown in Fig. 3, S100A2 significantly ($P < 0.05$) increased Ca^{2+} transient amplitude (360/380 from 0.26 ± 0.02 in control myocytes to 0.35 ± 0.03 in AdS100A2 myocytes, Fig. 3A, C). Moreover, S100A2 accelerated the kinetics of Ca^{2+} transient as shown by shortened decay time (time from peak to 50% decay, T50%, from 320.8 ± 17.7 ms in control myocytes to 274.4 ± 7.6 ms in AdS100A2 myocytes, T75% from 618.3 ± 42.8 ms to 499.4 ± 16.1 ms, and T90% from 1137.1 ± 66.5 ms to 965.3 ± 44.6 ms, $P < 0.05$, Fig. 3B, D) and faster Ca^{2+} transient increase velocity and decline velocity (Fig. 3E–F). S100A6 did not significantly alter Ca^{2+} transient amplitude or kinetics, which is consistent with its lack of effect on myocyte contractility (Fig. 3). These are evidence that S100A2, but not S100A6, positively regulates intracellular Ca^{2+} release and reuptake.

3.3. Mechanisms underlying S100A2 effects on cardiac Ca^{2+} cycling

To further determine the mechanism by which S100A2 increases systolic Ca^{2+} release during EC coupling, we monitored the spontaneous SR Ca^{2+} release events (Ca^{2+} sparks) in myocytes by using live cell confocal imaging (Fig. 4). Expression of S100A2 significantly increased Ca^{2+} spark frequency (from 1.6 ± 0.3 sparks per second per 100 μm in control myocytes to 3.1 ± 0.4 sparks per second per 100 μm in S100A2 myocytes, $P < 0.001$, Fig. 4B) providing evidence of increased RyR opening probability in intact myocytes. Also, S100A2 shortened the Ca^{2+} spark decay time (Fig. 4E) suggesting it hastens RyR closure after Ca^{2+} release. The effects of S100A2 on Ca^{2+} spark frequency and kinetics were similar to S100A1 (Fig. 4). Further, measurement of caffeine-induced Ca^{2+} release showed similar SR Ca^{2+} store in S100A2 expressing myocytes and control (Fig. 5). Therefore, increased fractional SR Ca^{2+} release during electrical stimulation is mainly associated with increased activity of RyR but not increased SR Ca^{2+} store. Further, the faster termination of RyR Ca^{2+} release by S100A2 may contribute to the accelerated Ca^{2+} transient decay. A direct interaction between S100A1 and RyR complex has been proposed [38], which could be the same mechanism underlying the effect of S100A2 on RyR.

As S100A1 has been shown to modulate SERCA2a and potentially NCX function [38–40], we tested whether the accelerated Ca^{2+} transient decay by S100A2 could be linked to these Ca^{2+} transporting proteins. However, the decay of caffeine-induced Ca^{2+} transient was unaltered by S100A2 or S100A6 in the presence (Fig. 5D) or absence (Fig. 5E–F) of extracellular Na^+ and Ca^{2+} , providing evidence of unchanged Ca^{2+} removal by NCX and SERCA. Moreover, expression of key Ca^{2+} handling proteins including SERCA2a, CSQ, PLN and NCX was unchanged after S100A2 or S100A6 expression (Fig. S3). Taken together, the data do not support a direct impact of S100A2 on SERCA or NCX.

Next, to determine whether the results of S100A2 in rodent myocytes can be extended to myocytes with human-like Ca^{2+} handling properties, we tested the effect of S100A2 in canine cardiac myocytes. Canine is a large animal that, in contrast to rodents, bears high similarities with humans in terms of heart anatomy, physiology, and cardiac Ca^{2+} handling properties [41]. Consistent with the findings in rat myocytes, S100A2 markedly enhanced contractility and accelerated relaxation in a frequency-dependent manner (Fig. S4). The fact that S100A2 conferred faster relaxation at higher pacing frequency (1 Hz), the range close to human heart rate, is particularly important for its potential application on patients.

3.4. S100A2 rescues contractile and Ca^{2+} handling deficits in failing myocytes

We tested whether S100A2 can mitigate myocyte functional deficits in failing myocytes by using a HF model through chronic ligation of the left anterior descending artery (LAD) of the rat heart [36]. Two to four weeks after LAD ligation, rats showed symptoms of heart failure and decreased cardiac contractile performance by echocardiography [36]. Myocytes from heart failure rats showed markedly decreased contraction amplitude, which was nearly doubled by S100A2 (SL shortening from $4.5 \pm 0.4\%$ in HF myocytes to $8.9 \pm 1.0\%$ in S100A2 expressing myocytes, $P < 0.05$, Fig. 6A). In this model of HF, myocyte relaxation dysfunction was not significant. Nonetheless, S100A2 was able to significantly accelerate relaxation in both normal and failing myocytes ($P < 0.05$, Fig. 6B). Enhanced Ca^{2+} release and cycling underlie the positive inotropic and lusitropic effects of S100A2, since S100A2 increased both Ca^{2+} transient amplitude and decay rate in HF myocytes ($P < 0.05$, Fig. 6C, D).

4. Discussion

S100 superfamily proteins have marked and diverse functions in biology. The potential application of S100 proteins to cardiac muscle function, however, remains under-developed. In the present study, we uncovered distinct functional outcomes among S100 Ca^{2+} binding protein family members in directly modulating cardiac Ca^{2+} dynamics. Expression of S100A2, a S100 family member present in human but not rodent hearts, significantly stimulated cardiac Ca^{2+} cycling to enhance contractility of adult rat and canine cardiac myocytes. These effects of S100A2 largely mimic those of S100A1 [23, 39, 42], the predominant cardiac S100 isoform. Interestingly, upon cardiac gene transfer S100A2 primarily localized to the cytosol and not nucleus, where it normally exists in other tissues of large animals functioning as a gene expression regulator [43]. In comparison, another S100 family member, S100A6, did not affect cardiac Ca^{2+} handling or contractility. Although S100A6 is highly homologous to S100A1 and S100A2, the variation in the hinge region and C terminus of this isoform is more divergent (Fig. 1A), and could account for differential biological function.

The S100 proteins are widely expressed throughout the body and broadly participate in intracellular signaling in diverse tissues [13, 14]. S100A1 is the predominant isoform expressed in the heart and has been shown to critically regulate cardiac Ca^{2+} cycling during EC coupling [19, 38]. The clinical relevance of S100A1 is shown by the finding that S100A1 expression level increases during hypertrophy and decreases in failing heart [44],

and supplementing S100A1 can ameliorate contractile dysfunction [23, 37, 42, 45]. However, S100A1 plays multi-functional roles beyond Ca^{2+} regulation in cardiac muscle and also promotes neurodegenerative diseases [22], which may potentially compromise its utilization in a clinical context. In this context, it is important to investigate the role of other S100 family members and homologues of S100A1 in heart physiology and pathophysiology. Through evaluating the effects of S100A2 and S100A6 in adult cardiac myocytes, we show for the first time that the expression of S100A2 isoform can also modulate cardiac Ca^{2+} handling and benefit cardiac performance. The fact that S100A2 is absent in rodent heart and expressed at low levels in human heart makes S100A2 gene transfer a potential new therapeutic approach to remediate contractile defects in failing myocytes.

Based on general sequence homology, a priori we hypothesized that S100A2 and S100A6 would similarly impact cardiac Ca^{2+} cycling and contraction. S100A2 and S100A6 share many features both structurally and functionally. They belong to the same subgroup in S100 family with the closest calculated phylogenetic distance indicating very close evolutionary relationship [46]. The three-dimensional structure of Ca^{2+} free S100A2 shows marked similarities with S100A6 [47]. Further, both S100A2 and S100A6 bind Ca^{2+} with moderate affinity, and Zn^{2+} with high affinity, and both are involved in gene expression and tumorigenesis [46]. However, differences do exist between S100A2 and S100A6, especially in the hinge region and C terminus, and in their different subcellular location, which may underlie the distinct functional outcomes. The location of endogenous S100A2 in many tissues is the nucleus, where a relatively high Zn^{2+} environment can significantly suppress its Ca^{2+} binding up to 300 fold [48]. However, when ectopically expressed in the rat myocytes, S100A2 remains in the cytosol, where the Zn^{2+} level is much lower. Therefore, increased Ca^{2+} binding affinity of S100A2 may occur. Ca^{2+} binding by S100A2 potentially leads to a larger conformational change to facilitate intermolecular interactions and an increase in putative Ca^{2+} buffering capacity. In the case of S100A6, a cytosolic protein, overexpression in cardiac myocytes does not alter its intracellular distribution and may not change its Ca^{2+} binding affinity. A recent study also indicates that binding with Ca^{2+} induces a more significant conformational change in S100A2 than in S100A6 [49]. The absence of detectable effects of S100A6 expression on myocyte function leaves open the question of the possible physiological role of endogenous S100A6.

It is interesting to speculate that cytosolic S100A2 would interact with a new set of proteins, which do not usually have access to the nuclear-located S100A2 in other tissues. Our Ca^{2+} spark data indicate significant functional consequences of S100A2 on the Ca^{2+} release channel, RyR, an effect similar to S100A1 [21]. However, unlike S100A1, which also influences SERCA2a and NCX function [39, 40], S100A2 is unlikely to directly influence SERCA2a or NCX function, because the decay of caffeine-induced Ca^{2+} transient is unaltered. As discussed above, a significant increase in Ca^{2+} binding affinity due to the absence of Zn^{2+} inhibition may cause conformational changes in S100A2 and subsequent interaction with new targets in the cytosol. Further studies on the molecular basis of the structural and functional alterations of S100A2 may identify the interaction of S100A2 with RyR Ca^{2+} release channel and other proteins. For example, whether S100A2 can, like S100A1, regulate myofilament [42] and mitochondrial [50] functions are interesting questions for future studies.

S100A2 significantly ameliorated depressed Ca^{2+} handling and contractility in failing myocytes. Importantly, both contractile and relaxation functions are almost doubled by S100A2 expression, an effect much stronger than in control healthy myocytes and comparable to that of S100A1 in failing myocytes [23]. Current heart failure therapy focuses on either increasing contractility (e.g. positive inotropic agents) or accelerating relaxation. Developing a single therapy dealing with both systolic and diastolic dysfunction is intriguing as many heart failure patients suffer both defects. Additionally, the low expression level of S100A2 in normal heart [25] implies fewer endogenous targets that associate with side effects on other signaling pathways of the myocyte. Moreover, the normal nuclear localization of S100A2 in myocytes of larger animals is linked to a gene transcription regulation role, which is unlikely affected by overexpression of S100A2, because the overexpressed protein remains in cytosol. Overall, S100A2 gene transfer could be a new approach for heart failure gene therapy. Further studies on the *in vivo* gene delivery will be highly informative and crucial for advancing S100A2 gene transfer to the preclinical stage.

In summary, we show here that S100 family member S100A2 (but not S100A6), which shares marked structural similarities to S100A1, enhances Ca^{2+} cycling to improve contraction and relaxation performance in normal and failing cardiac myocytes. Unlike its role in gene expression in other tissues, S100A2 is present in the cytosol and appears to directly affect cardiac Ca^{2+} handling. Gene transfer of S100A2 may also benefit other systems that rely on timely and robust Ca^{2+} handling, such as nervous system, skeletal muscles and smooth muscles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HF	heart failure
Ca^{2+}	calcium
SERCA2a	sarcoplasmic reticulum Ca^{2+} ATPase 2a
RyR	ryanodine receptor Ca^{2+} release channel
CSQ	calsequestrin
PLN	phospholamban
NCX	$\text{Na}^+/\text{Ca}^{2+}$ exchanger
SL	sarcomere length

LAD left anterior descending artery.

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Highlights

- We found distinct functions of highly homologous S100 family members
- S100A2, but not A6, increased cardiac Ca^{2+} transients and contractility
- S100A2 enhanced SR Ca^{2+} release from RyR
- Ectopic expression of S100A2 ameliorated defective contraction in failing myocytes

Fig. 1. Adenovirus-mediated S100A2 or S100A6 expression in adult cardiac myocytes
A, Sequence alignment of S100 proteins. The S100 four α -helices are highlighted in red. B, Schematic of the adenovirus vector expressing S100A2 or S100A6. C, Immunofluorescent images showing the uniform expression of S100A2 and S100A6 in >95% cardiac myocytes 3 days after gene transfer. Red: anti-S100A2 or anti S100A6; green: anti-actin. Scale bars = 100, 10 or 2 μ m for different magnifications (left to right). D, Western blots demonstrating time dependent expression of S100A2 and S100A6 determined by specific antibodies against S100A2 or S100A6, respectively. Data are representative of at least 3 experiments.

Fig. 2. Increased myocyte contraction amplitude and relaxation speed by S100A2, but not S100A6

A–B, Representative original sarcomere length (SL) traces (A) and normalized SL traces (B) from control myocytes and myocytes expressing S100A2 or S100A6 after 3 days of gene transfer. The myocytes were also loaded with fura-2 for measurement of Ca^{2+} transients in Fig. 3. C–F, Summarized SL shortening amplitude (C), decay time course (D, Tp: time to peak; T50%, T75% and T90%: time from peak to 50%, 75% and 90% relaxation, respectively), contraction velocity (E), and relaxation velocity (F) of control myocytes and

myocytes expressing S100A2 or S100A6. Data are mean $\pm$ SEM, n = 25–40 cells from 3–4 rats. *, $P < 0.05$ versus Control.

Fig. 3. Increased Ca^{2+} transient amplitude and faster decay in cardiac myocytes by S100A2, but not S100A6

A–B, Representative original (A) and normalized (B) Ca^{2+} transient traces from control myocytes and myocytes expressing S100A2 or S100A6 after 3 days of gene transfer. C–F, Summarized Ca^{2+} transient amplitude (C), decay time course (D, Tp: time to peak; T50%, T75% and T90%: time from peak to 50%, 75% and 90% decay, respectively), increase velocity (E) and decay velocity (F) of control myocytes and myocytes expressing S100A2 or S100A6. Data are mean $\pm$ SEM, $n = 26$ –40 cells from 3–4 rats. *, $P < 0.05$ versus Control.

Fig. 4. Effect of S100A2 expression on spontaneous Ca^{2+} sparks

A, Representative confocal line-scan images of Ca^{2+} sparks detected in intact control myocytes and myocytes expressing S100A1 or S100A2 after 3 days of gene transfer. B–E, Summarized Ca^{2+} spark frequency (B), amplitude ($\Delta F/F_0$, C) rise time (D) and decay time (E) in control and S100A1 or S100A2 expressing myocytes. Data are mean $\pm$ SEM, $n = 121$ –239 sparks in 30–38 cells from 4 rats. *, $P < 0.05$ versus Control. $\dagger$, $P < 0.001$ versus Control.

Fig. 5. Effect of S100A2 expression on caffeine-induced Ca²⁺ transients

A–B, Representative traces of caffeine-induced Ca²⁺ transients from control myocytes (A) and myocytes after 3 days of infection with AdS100A2 (B). C–D, Summarized data of Ca²⁺ transient amplitude (C) and decay kinetics (T75%: time from peak to 75% decay, D) of control myocytes and myocytes expressing S100A2 or S100A6. Data are mean ± SEM, n = 8–18 cells from 3–4 rats. E–F, Summarized data of caffeine-induced Ca²⁺ transient amplitude (E) and decay kinetics (F) of control and S100A2 expressing myocytes in the absence of extracellular Na⁺ and Ca²⁺. Data are mean ± SEM, n = 9 cells from 2 rats.

Fig. 6. Improved contraction and Ca^{2+} handling in failing myocytes by S100A2

A, Summarized SL shortening in normal and failing myocytes (HF) with or without S100A2 gene transfer. B, Summarized relaxation velocity of normal and HF myocytes with or without S100A2 gene transfer. Data are mean $\pm$ SEM, $n = 17$ – 27 cells for each group. *, $P < 0.05$ versus Control. C, Summarized Ca^{2+} transient amplitude of HF myocytes with or without S100A2 gene transfer. D, Summarized Ca^{2+} transient decay kinetics (T25%, T50%, T75% and T90%: time from peak to 25%, 50%, 75% and 90% decay, respectively). Data are mean $\pm$ SEM, $n = 6$ – 7 cells. *, $P < 0.05$ versus HF.