Journal of Molecular and Cellular Cardiology xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

S100A1 gene therapy for heart failure: A novel strategy on the verge of clinical trials

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ARTICLE INFO

Article history:
Received 10 June 2010
Received in revised form 11 August 2010
Accepted 13 August 2010
Available online xxxx

Keywords:
S100A1
Gene therapy
Heart failure
Calcium
Sarcoplasmic
Reticulum
Myofilaments
Mitochondria

ABSTRACT

Representing the common endpoint of various cardiovascular disorders, heart failure (HF) shows a dramatically growing prevalence. As currently available therapeutic strategies are not capable of terminating the progress of the disease, HF is still associated with a poor clinical prognosis. Among the underlying molecular mechanisms, the loss of cardiomyocyte Ca^{2+} cycling integrity plays a key role in the pathophysiological development and progression of the disease. The cardiomyocyte EF-hand Ca^{2+} sensor protein S100A1 emerged as a regulator both of sarcoplasmic reticulum (SR), sarcomere and mitochondrial function implicating a significant role in cardiac physiology and dysfunction. In this review, we aim to recapitulate the translation of S100A1-based investigation from first clinical observations over basic research experiments back to a near-clinical setting on the verge of clinical trials today. We also address needs for further developments towards "second-generation" gene therapy and discuss the therapeutic potential of S100A1 gene therapy for HF as a promising novel strategy for future cardiologists.

Contents

1. Introduction

Heart failure (HF) is approaching epidemic proportions in industrialized societies and this trend will undoubtedly continue in relentlessly aging populations. The mortality rate in this syndrome,

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0022-2828/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2010.08.012

which represents the common endpoint of various cardiovascular disorders, is about 50% per year in advanced stages [1]. Despite the recognized success of the current pharmacological arsenal in the treatment of HF, including β-adrenergic receptor blockade, angiotensin-converting enzyme inhibitors and aldosterone antagonists, the progress of the disease appears to be unstoppable, inevitably leading to death either due to pump failure or arrhythmias [2].

With increasing knowledge of basic molecular mechanisms involved in the development and progression of HF, the possibility of specifically targeting pathophysiological key players is evolving at present. Of note, advanced insight into underlying pathomechanisms, gleaned from pre-clinical animal models, has provided a rationale for the methodical approach to reverse human HF by directly manipulating the genetic structure of the cardiomyocyte. Thus, gene therapy for HF has emerged as a promising therapeutic strategy, potentially extending the therapeutic spectrum of clinical cardiology in the near future [3].

Much evidence has accumulated that abnormal regulation of cardiomyocyte Ca^{2+} homeostasis represents a pathophysiological key event in the two main causes of death in HF patients: 1) myocardial pump failure and 2) ventricular arrhythmias [4,5]. Reversing dysfunctional cardiomyocyte Ca^{2+} handling might therefore bear the potential of an effective therapeutic intervention, serving as a viable add-on to conventional therapies that mitigate secondary maladaptive events in the first line [6]. After almost two decades of pre-clinical investigations, first clinical gene therapy trials aiming at aberrant cardiomyocyte Ca^{2+} cycling in chronic HF are currently underway. As first results already indicate safety, feasibility and potential clinical benefits [7], these studies will be of critical importance for further promising gene targets that are on the verge of first-in-human trials today.

This review details the impact of the EF-hand Ca^{2+} sensor protein S100A1 on cardiomyocyte Ca^{2+} homeostasis and the subsequent regulation of excitability, contractile performance, energy metabolism, hypertrophic growth and cell survival [8–10]. Approaching this issue from a translational point of view, we will initially deal with first clinical observations and experimental in vitro studies of S100A1's molecular interactions, before focussing on rationale and proof-of-concept for S100A1 gene-based HF therapy in small- and medium-size animal models. Finally, we will recapitulate the translation of experimental results into pre-clinical large animal models and human failing cardiomyocytes and discuss the therapeutic potential of S100A1 gene therapy as a promising strategy in the combat against HF.

2. Clinical pathology: point of origin for exploring S100A1 cardiovascular biology

2.1. Abnormal S100A1 expression in failing myocardium

Predominant expression of the EF-hand Ca^{2+} sensor protein S100A1 in normal human myocardial tissue conceptualized the hypothesis of a specific S100A1 function in cardiovascular physiology (previously reviewed in detail in [8–12]). The almost exclusive abundance in cardiomyocytes subsequently became the point of origin for a comprehensive analysis of S100A1 expression levels in diseased human hearts. Assaying both mRNA and protein levels in ischemic and dilated cardiomyopathy unveiled diminished S100A1 transcript and protein levels as a common pathological pattern [13]. Continuative studies in several experimental HF models – ranging from small (rat and mouse) [14–17] over medium-sized (rabbit) (Most Patrick, unpublished results) to large animals (pig and dog) [18,19] – revealed the same molecular abnormality found in humans. Additionally, non-invasive and invasive assessment of cardiac performance in animal HF models indicated an inverse relationship between cardiomyocyte S100A1 expression and the severity of the disease [16], further supporting the notion of abnormal S100A1 expression as a pathomolecular hallmark of HF across species.

2.2. Pathomechanisms governing S100A1 downregulation in heart failure

The altered myocardial S100A1 expression pattern in HF was considered as an immanent part of adverse fetal gene reprogramming [16,17], resembling the low S100A1 expression levels during fetal heart development [20]. Indeed, further studies in cultured rat cardiomyocytes corroborated this pathophysiological association. Along with hypertrophic growth, chronic stimulation of cardiomyocytes with angiotensin II, endothelin 1, phenylephrine and protein kinase C (PKC) agonists resulted in a time- and dose-dependent suppression of S100A1 mRNA and protein levels [16]. Ensuing S100A1 promoter analyses in rodents revealed several negative regulatory motifs controlled by inhibitory transcription factors downstream of G-protein coupled receptors and PKC, thereby providing a plausible explanation for S100A1's apparent susceptibility to maladaptive growth [20]. Conclusively, downregulation of the cardiomyocyte EF-hand Ca^{2+} sensor protein S100A1 in failing myocardium in vivo and in vitro indicated potential clinical relevance and thus prompted the experimental studies aiming at deciphering S100A1's role in normal and diseased myocardium.

3. Molecular and cellular studies: discovering S100A1's impact on cardiomyocyte Ca^{2+} homeostasis

3.1. S100A1 molecular characteristics

S100A1 predominantly exists as a symmetric, antiparallel dimer of two monomeric subunits, whereas the dimeric structure is favored even at picomolecular monomer concentrations. The monomer consists of two EF-hand Ca^{2+} binding motifs (an N-terminal non-canonical and a C-terminal canonical sequence) interconnected by an intermediate region (hinge region). Upon Ca^{2+} binding to both EF-hands, S100A1 undergoes a major conformational change in the C-terminal EF-hand leading to the exposure of a concave hydrophobic pocket in each monomer. The pocket is considered to be critically involved in Ca^{2+}-dependent recognition of S100A1 target proteins. Ca^{2+} binding to S100A1 occurs with a Kd of ≈ 200–500 μM at the N-terminal and a Kd ≈10–50 μM at the C-terminal site. Furthermore, the Ca^{2+} affinity is tightly regulated by redox- and NO-dependent posttranslational modifications. For a more detailed insight into S100A1's molecular characteristics, we kindly refer the reader to related reviews elaborating on this point [8–12].

3.2. Targeting sarcoplasmic reticulum Ca^{2+} handling

Taking advantage of adenoviral technologies, experimental strategies first focussed on functional alterations in response to overexpression of human S100A1 protein in rat and rabbit ventricular cardiomyocytes. Under electrical field stimulation, both systolic and diastolic contractile properties were improved and analysis of cytosolic Ca^{2+} transients revealed increased systolic amplitudes and accelerated diastolic Ca^{2+} removal [21–24]. Interestingly, this basal gain-in-function was preserved and additive to stimulation with β-adrenergic receptor agonists but neither relied on nor changed downstream cAMP-dependent signaling including protein kinase A (PKA) and calmodulin-dependent kinase II (CaMKII) [21–24]. Continuative studies in rat engineered heart tissue (EHT) enabled insight into the impact of elevated S100A1 protein concentrations on in vitro cardiomyocyte performance under loaded conditions. Adenoviral-mediated S100A1 overexpression in EHTs improved maximal as well as length- and frequency-dependent isometric force generation, closely resembling Frank–Starling-mechanism and force–frequency-relationship of intact hearts. As observed in isolated cardiomyocytes,

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the S100A1-mediated gain-in-function of EHTs was preserved under β-adrenergic receptor stimulation without affecting cAMP-dependent kinase activities [22,25].

Analyses in chemically skinned cardiomyocytes with intact sarcoplasmic reticulum provided first evidence for a dose-dependent stimulatory effect of S100A1 on sarcoplasmic reticulum (SR) Ca^{2+} uptake suggesting enhanced SR Ca^{2+} ATPase (SERCA2) activity [22,24]. Accordingly, both cardiomyocytes with viral-based S100A1 overexpression and those isolated from transgenic mice with elevated cardiomyocyte S100A1 expression levels exhibited increased SR Ca^{2+} contents under steady-state conditions [22,24,26]. Vice versa, siRNA-based S100A1 gene silencing in neonatal rat cardiomyocytes resulted in an opposing phenotype characterized by diminished Ca^{2+} transients and SR Ca^{2+} load under basal conditions and β-adrenergic receptor stimulation [21]. Further studies focusing on SR Ca^{2+} release mechanisms additionally unveiled an inhibitory effect of S100A1 on both rate and amplitude of elementary Ca^{2+} events (Ca^{2+}-sparks) in skinned cardiomyocytes, thereby reducing spontaneous diastolic SR Ca^{2+} release [27]. In line with these observations, subsequent experiments eventually demonstrated decreased frequency and amplitude of Ca^{2+} sparks along with increased SR Ca^{2+} load in quiescent rat cardiomyocytes with viral-based overexpression of human S100A1 [28]. These results mechanistically attribute both accelerated diastolic SR Ca^{2+} uptake and diminished spontaneous SR Ca^{2+} release to enhanced diastolic SR Ca^{2+} storage, which most likely accounts in part for S100A1's Ca^{2+}-dependent inotropic effects. First evidence for a direct stimulatory effect of S100A1 on systolic SR Ca^{2+} release, on the other hand, came from studies employing voltage-clamped cardiomyocytes, facilitating a comprehensive assessment of transsarcolemmal and transsarcoplasmic Ca^{2+} fluxes. Here, intracellular instillation of S100A1 protein via patch pipette neither altered L-type Ca^{2+} channel activity nor reverse or forward sodium–calcium exchanger (NCX) mode in cardiomyocytes, but enhanced systolic SR Ca^{2+} release and Ca^{2+} transient amplitudes [29]. Enhanced Ca^{2+}-induced SR Ca^{2+} release (CICR) was also evident in SR vesicle preparations from S100A1 transgenic mice and those from wild type animals when treated with recombinant S100A1 protein [16,26]. In line with these data, 3H-ryanodine binding was enhanced in SR vesicles when exogenous S100A1 was added in the presence of supra-diastolic free Ca^{2+} concentrations (i.e. Ca^{2+} concentrations exceeding 300 nM) [15].

On the molecular level, S100A1 was found to interact with the SR Ca^{2+} release channel/ryanodine receptor (RyR) [15,16,26,27,29–32] and the SR Ca^{2+} ATPase/phospholamban complex (SERCA2/PLN) [15,16,29,33]. Interaction of S100A1 with both SR targets appeared to be Ca^{2+}-dependent and mediated by the S100A1 carboxy-terminus (S100A1ct). According to previously reported Ca^{2+}-dependent changes in S100A1's tertiary structure [34], the hydrophobic S100A1ct epitope is considered as one of the binding domains conveying the functional effects of S100A1 in cardiomyocytes. Indeed, a synthetic S100A1ct peptide was sufficient to mimic effects of the native Ca^{2+}-activated protein in skinned cardiomyocyte preparations, corroborating the functional importance of this domain [15,27]. Differential effects of S100A1 on systolic and diastolic RyR function might be attributable to previously reported three distinct binding sites at the RyR [31] of which one was identified as the calmodulin binding epitope [32]. Binding epitopes for S100A1 at SERCA2 or PLB, however, have not yet been identified. Taken together, although underlying molecular mechanisms are subject of ongoing research, various in vitro and in vivo studies highlighted S100A1's impact on both diastolic and systolic SR Ca^{2+} handling in cardiomyocytes, apparently resulting in a Ca^{2+}-dependent inotropic effect. As cardiomyocyte excitability and survival depend on balanced SR Ca^{2+} fluxes [4], beneficial consequences of increased S100A1 protein abundance might extend far beyond improved cardiomyocyte contractile performance, potentially even preventing Ca^{2+} triggered arrhythmias and cell death [28].

3.3. Modulating sarcomeric stiffness and Ca^{2+} responsiveness

Cardiac titin recently emerged as an additional molecular target of S100A1 [35,36]. Ranging from Z-disk to M-line, this giant protein is mainly responsible for the generation of passive tension within the sarcomere upon stretch [37]. Moreover, titin transiently interacts with F-actin through its PEVK domain, forming a molecular "vicious break" that seems to significantly contribute to diastolic myocardial stiffness [36]. In the presence of Ca^{2+}, S100A1 binding to several sites along titin's extensible region, including the PEVK domain, reduces the force that arises as F-actin slides relative to the PEVK domain [35]. In line with this molecular interaction, S100A1 attenuated passive tension development of stretched skinned left ventricular muscle strips in a Ca^{2+}-dependent manner indicating its modulatory effect on intact sarcomere compliance [36]. In this context, the Ca^{2+}-dependency of S100A1-mediated regulation of titin–actin interplay is of particular interest, because it might provide a mechanism to adequately switch off diastolic PEVK–actin interaction at systolic Ca^{2+} levels, thereby preventing a titin-based mechanical restraint to systolic sarcomeric shortening.

In addition, S100A1 protein was found to dose-dependently modulate myofilament Ca^{2+} responsiveness and cooperativity in skinned cardiac trabeculae without altering maximal force development and cAMP-dependent phosphorylation of troponins [22]. Outside the I-bands, S100A1 has also been detected at sarcomeric A-bands, the Z- and M-lines [38], but it is yet unknown whether S100A1 might interact with any other regulatory molecule associated with thin filament function. Employing synthetic S100A1 peptide fragments, however, unveiled that the effect of the native protein can sufficiently be mimicked by the S100A1ct peptide [39]. This observation might indicate the existence of a common molecular mechanism for S100A1-mediated regulation of both SR and sarcomeric function. Given the heightened systolic performance of S100A1-overexpressing myocardium, the effect of S100A1 on myofilament Ca^{2+} responsiveness seems to contribute to S100A1-mediated improvement of diastolic performance by facilitating the dissociation of thick and thin myofilaments. Hence, although the influence of S100A1 on myofilament function certainly warrants further investigation, several studies showed that S100A1 modulates both diastolic and systolic sarcomere performance, apparently adjusting myofilament function to S100A1-mediated improvement of cytosolic Ca^{2+} cycling.

3.4. Regulating mitochondrial function and energy metabolism

S100A1's mitochondrial location [21,40] prompted further exploration of possible molecular interactions and lead to the identification of several S100A1 binding targets within the inner mitochondrial membrane and matrix, including key molecules of high-energy phosphate production (F1-ATPase), cytosolic ATP translocation (adenine nucleotide translocator, ANT) and citric acid cycle dependent NADH generation (isocitrate dehydrogenase; IDH) [21]. Studies in neonatal rat S100A1-overexpressing cardiomyocytes provided evidence for enhanced adenosine triphosphate (ATP) levels under basal conditions [21]. An opposing phenotype was evident after siRNA-mediated S100A1 gene silencing in these cells with a 2–3 fold decrease in S100A1 protein, further illustrating the impact of S100A1 on cardiomyocyte energy homeostasis. Molecular studies then focused on the potential impact of S100A1 on the main site of ATP production, the mitochondrial ATPase. S100A1 was actually found to directly interact with the F1 subunit (α- and β-chain) of the mitochondrial ATPase in a Ca^{2+}- and pH-dependent manner [21]. The enhanced cellular ATP content observed in S100A1-overexpressing cardiomyocytes could therefore be attributed to a direct Ca^{2+}-dependent stimulatory effect of S100A1 on ATP-generating enzymes. These results also suggest that a Ca^{2+}-dependent interaction between S100A1 and mitochondrial ATPases

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could help to adjust cardiomyocyte ATP generation to augmented SERCA2 activity and improved myofilament sliding.

Moreover, Ca^{2+}-dependent S100A1 interaction with IDH and ANT may imply a regulatory role of S100A1 on mitochondrial function both up- and downstream of F1-ATPase prompting the speculation that S100A1 might relay enhanced cytosolic Ca^{2+} turnover to increased IDH activity and mitochondrial NADH generation as S100A1 apparently mediates an increase of mitochondrial Ca^{2+} uptake by a hitherto unknown mechanism (M. Boerries, unpublished results). Additionally, S100A1 might facilitate ANT-mediated cytosolic translocation of ATP, thereby potentially empowering the cardiomyocyte to meet an increased ATP demand caused by heightened SR and myofilament activity.

In summary, these data highlight S100A1's Ca^{2+}-dependent regulatory function in the metabolic network of cardiomyocytes. Although these results warrant further profound investigation, S100A1 could be considered as a coupling link between increased cytosolic ATP demand and enhanced mitochondrial energy supply. Figs. 1(A) and (B) summarize the conceptual framework of an S100A1 Ca^{2+}-dependently controlled metabolic network in cardiomyocytes.

4. Small animal heart failure models: rationale and proof-of-concept for S100A1 gene therapy

4.1. S100A1 transgenic and gene-ablated mouse models

The rationale for S100A1 gene-based therapeutic strategies targeting aberrant Ca^{2+} handling in failing cardiomyocytes originates from studies in S100A1 transgenic (STG) [26] and gene-ablated mouse (SKO) models [41–43], which presented opposing phenotypes in response to ischemic cardiac damage [14,16]. STG mice exhibited superior survival than controls, whereas SKO animals showed exaggerated early mortality, most likely due to reduced contractile performance and enhanced susceptibility to arrhythmias [14,16,41]. As expected, post-MI cardiac performance of STG mice was superior to wild type controls, obviously protecting from sympathetic hyperactivity, pathological myocardial remodeling and stress-induced cardiomyocyte apoptosis. In contrast, infarcted SKO hearts displayed accelerated left ventricular remodeling and transition to contractile failure with greater cardiomyocyte apoptosis. Consistently, maintained SR Ca^{2+} handling was found in STG cardiomyocytes while S100A1 deficiency led to to SR Ca^{2+} cycling abnormalities in SKO animals. Of note, SKO mice also exhibited a detrimental outcome in response to chronically elevated afterload induced by transaortic constriction [42]. In indicating the indispensability of S100A1 for the adaption of the heart to hemodynamic stress, these studies concurrently advocated therapeutic strategies to maintain or restore cardiac S100A1 expression. In light of S100A1's multifaceted molecular profile, such molecular interventions could potentially prevent or reverse maladaptive cardiomyocyte Ca^{2+} handling, which accounts for arrhythmias, dysfunctional energy homeostasis, maladaptive growth, apoptosis and pump failure.

4.2. Viral-based S100A1 overexpression in cardiomyocytes in vivo

Experimental proof-of-concept for viral-based S100A1 HF gene therapy originates from studies utilizing catheter-mediated cardiac-targeted S100A1 gene delivery in small animal HF models [15,44–46]. First studies utilized adenoviral vectors with S100A1 under control of a cytomegalievirus promoter, administered via coronary arteries shortly after acute myocardial infarction in rats [46]. This strategy, resulting in an approximately 30–40% transfection rate of remote myocardium, was proven to be highly effective in preventing postischemic deterioration of left ventricular performance along with attenuation of cardiac remodeling. A subsequent study testing adenoviral-based S100A1 gene therapy in the context of chronic HF in rats revealed for the first time the profound therapeutic potency of S100A1 gene delivery, providing proof-of-concept for an almost complete rescue of functional cardiac performance within a 2 week follow-up period [15]. Consistent with S100A1's molecular framework, the same study demonstrated a series of expected and even unexpected therapeutic effects in vivo, ranging from restored cardiomyocyte SR Ca^{2+} handling and energy homeostasis and reversed cardiac remodeling to reconstituted sodium levels and improved βAR-responsiveness. In an attempt to assess long-term therapeutic efficiency of S100A1 gene therapy, a continuative study, employing intracoronary adeno-associated (AAV) S100A1 gene delivery under control of a cardiomyocyte-specific promoter, unveiled favorable long-term therapeutic effects, *recapitulating* previous functional and structural in vitro and in vivo beneficial findings [44].

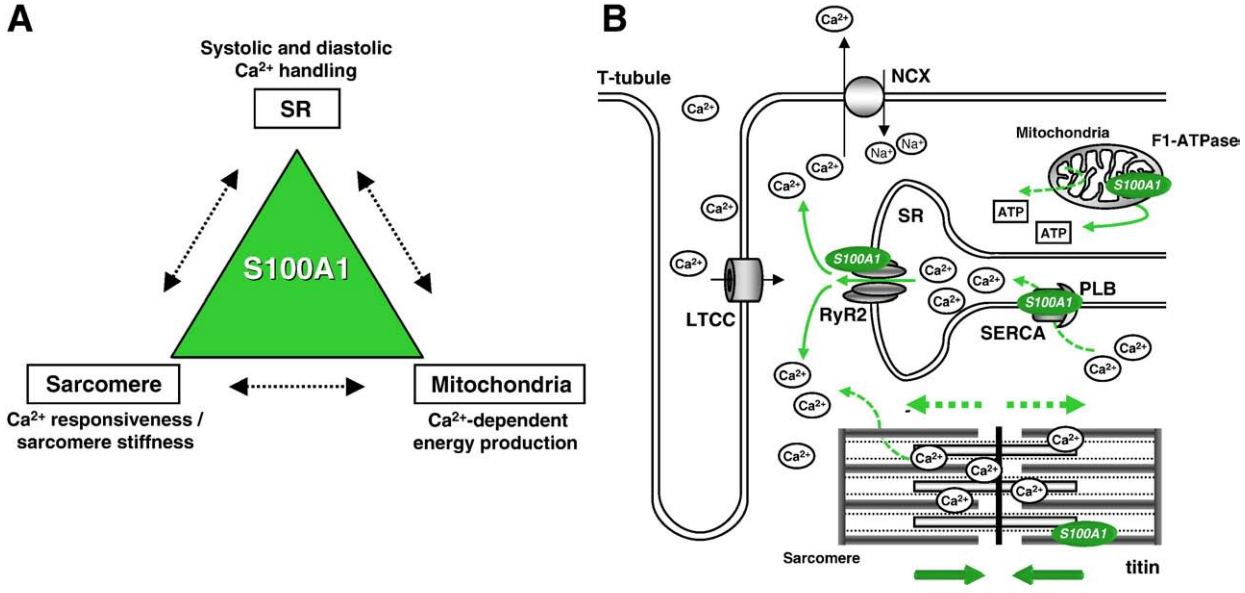

Fig. 1. (A) Intracellular S100A1 regulates and connects function of three Ca^{2+}-driven key subcellular compartments in ventricular cardiomyocytes: the sarcoplasmic reticulum, the sarcomere and mitochondria. (B) Functionally validated molecular targets modulated by Ca^{2+}-activated S100A1 comprise RyR, SERCA2/PLB, cardiac titin and mitochondrial ATPase. Bold and dotted arrows indicate S100A1 effects during systole and diastole, respectively (reproduced from Voelkers et al. J Biomed Biotechnol. 2010;2010: 178614).

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Of note, the same study compared efficiency of AAV-based S100A1 gene therapy with chronic βAR blockade by metoprolol alone as well as with a combined treatment. Strikingly, S100A1-gene-based HF treatment exhibited superior effectiveness than metoprolol alone and combined treatment unveiled synergistic effects of S100A1 overexpression and metoprolol on myocardial remodeling [44]. Molecular mechanisms and effects conveying S100A1's therapeutic actions are summarized in Fig. 2. Once Ca^{2+} handling is restored, treated myocardium might chronically exert a hyper-contractile state functionally compensating for myocardium lost due to ischemic or other damage. Based on insight in transgenic animals with different amounts of myocardial S100A1 expression (1.5–6 fold) where levels of S100A1 positively correlated with the chronic inotropic state of hearts (P. Most, unpublished results), residual levels of S100A1 in diseased myocardium are expected to determine long-term contractile performance.

4.3. Limitations of proof-of-concept studies inherent to small animal heart failure models

Although intriguing, these studies, being conducted in mice and rats, raise the important question: "Can we really extrapolate those discoveries to humans and predict clinical efficiency?" The clear answer is: "No, we can't." This conclusion takes into account fundamental differences in physiological key characteristics between rodents and humans. Striking differences in heart rate, adrenergic receptor ratios, oxygen consumption and metabolism as well as in cellular Ca^{2+} handling, electrophysiology, myofilament composition

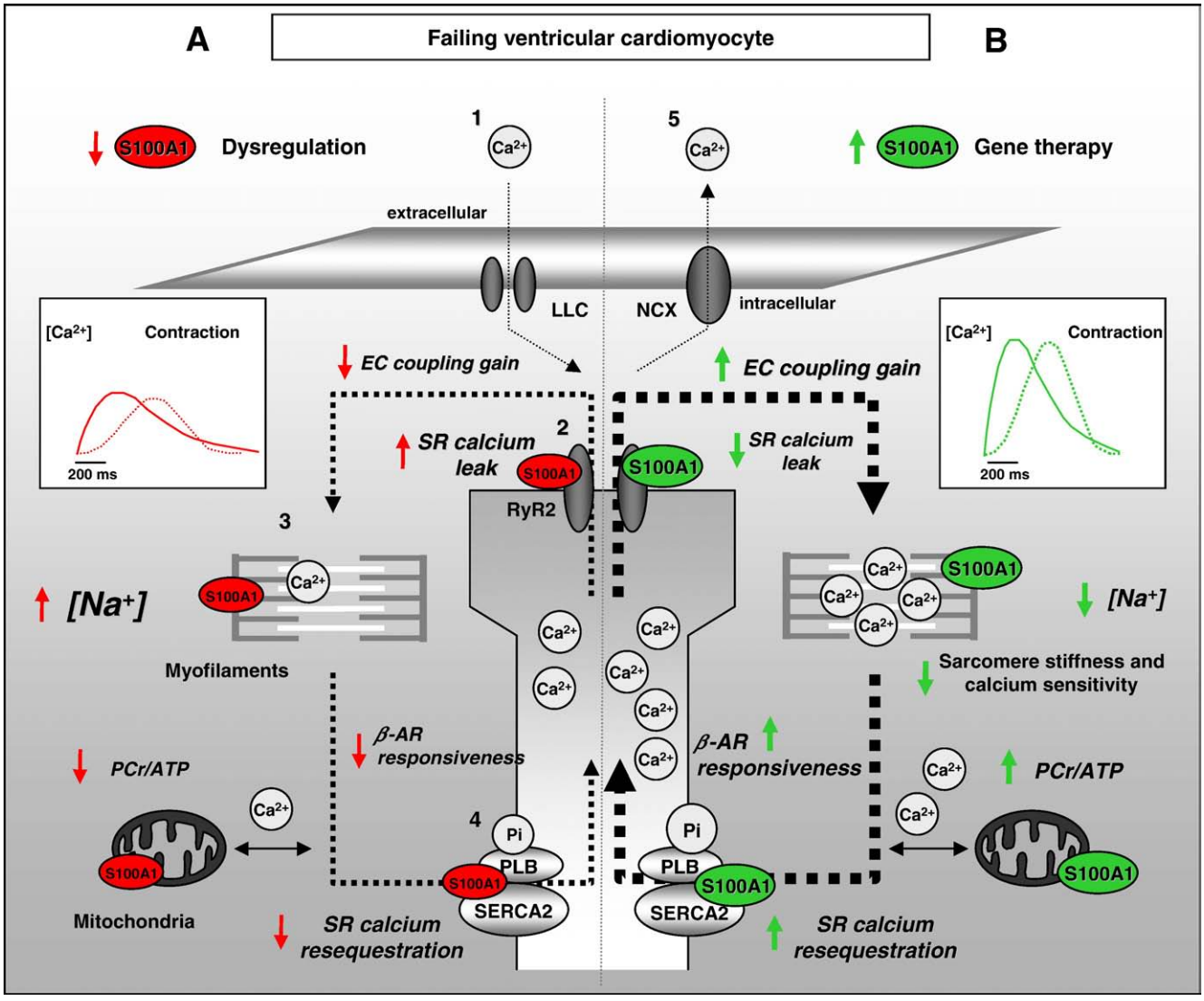

Fig. 2. Mechanisms conveying S100A1 therapeutic actions in failing ventricular cardiomyocytes. (A) 1–5: Excitation contraction (ec) coupling to occur requires (1) action potential dependent systolic opening of the L-type calcium channel (LLC) enabling a transsarcolemmal Ca^{2+} entry (2) triggering sarcoplasmic reticulum (SR) Ca^{2+} release via the ryanodine receptor 2 (RyR2) that in turn (3) activates myofilament cross-bridge cycling and mechanical force development. During diastole, (4) SR Ca^{2+} resequestration occurs through the SR Ca^{2+} ATPase (SERCA2) allowing Ca^{2+} dissociation from myofilaments and relaxation to occur. (5) Under steady-state conditions, the sodium–calcium exchanger (NCX) exerts balanced extrusion of LLC mediated Ca^{2+} entry. Failing ventricular cardiomyocytes displaying decreased S100A1 abundance (red) show defective ec-coupling characterized by impaired ec-coupling gain, enhanced SR Ca^{2+} leak and diminished SR Ca^{2+} resequestration. Further hallmarks are impaired β-AR responsiveness, increased intracellular sodium concentrations and altered energy homeostasis. S100A1 interacts with both RyR2 and the SERCA2a/PLB complex (SR) and is present at myofilaments (cardiac titin) and mitochondria (F1-ATPase, IDH and ANT). (B) Proposed mechanistic model for S100A1's therapeutic actions. Restored/increased cardiomyocyte S100A1 protein levels result in improved ec-coupling gain and enhanced systolic SR Ca^{2+} release through hightened RyR2 but not LLC activity. Myofilament stiffness is decreased via S100A1/titin interaction accompanied by facilitated diastolic Ca^{2+} dissociation. Augmented systolic SR Ca^{2+} release is balanced by enhanced diastolic SERCA2a activity with unchanged PLB phosphorylation at serin-16 and threonin-17. Enhanced SERCA2a activity together with diminished diastolic RyR2 activity results in increased SR Ca^{2+} resequestration and diminished SR Ca^{2+} leak. Concurrently, sodium concentrations decrease and energy production and flux are improved. Inlets illustrate the net effect of diminished (red) and restored/increased cardiomyocyte S100A1 protein levels (reproduced from Kraus et at. J Mol Cell Cardiol. 2009 47(4):445–55).

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and mitochondrial function readily preclude extrapolation [47,48]. Therefore, large animal heart failure models, more closely approximating human anatomy and physiology, are essential for the translation of discoveries from basic research to a pre-clinical setting [47,48]. For this reason it appeared to be mandatory to assess therapeutic safety and efficiency of S100A1 gene-based therapeutic strategies in large animal models recapitulating common clinical key features of human heart failure. In addition, large animal HF models not only imply the challenge of "scalability" but also can actually provide a true estimation of feasibility under near-clinical conditions. Furthermore, complementary studies employing failing human cardiomyocytes might be desirable to actually predict therapeutic efficiency in human failing myocardium.

5. Pre-clinical animal models and human heart failure: predicting clinical safety and efficiency of S100A1 gene therapy

5.1. Therapeutic feasibility of S100A1 gene therapy in postischemic pigs and human failing cardiomyocytes

Employing AAV-S100A1 vector technology with proven cardiomyocyte-targeted expression in rodents, a study in a postischemic pig HF model revealed first evidence for feasibility and, most importantly, long-term therapeutic efficiency in a pre-clinical setting [19]. Myocardial infarction was established by percutaneous catheter-based occlusion of the left circumflex artery (LCX), yielding a large-animal model that recapitulates the clinical characteristics of myocardial infarction and HF. Cardiac-targeted delivery of AAV-S100A1 was accomplished 2 weeks post-MI via the anterior cardiac vein with concurrent temporary blockade of the descending left coronary artery (LAD). Comprehensive expression analyses and assessment of clinical chemistry and blood count after 2 months indicated cardiac-restricted transgene expression without any signs of extra-cardiac abnormalities. Compared to AAV-luciferase treated animals, S100A1 gene therapy exerted long-term improvement of non-invasively and invasively measured systolic and diastolic cardiac indices, reversed cardiac remodeling and restored surrogate markers of cardiac energy homeostasis. Interestingly, restoration of cardiac performance was also evident in response to βAR stimulation and analyses of isolated cardiomyocytes unveiled reconstituted cytosolic Ca^{2+} handling in S100A1-treated hearts. Complimentary, an in vitro study on isolated human failing cardiomyocytes provided first evidence for the therapeutic efficiency of S100A1 in human failing myocardium (Henriette Brinks, unpublished data). Restoration of S100A1 protein concentration by adenoviral gene delivery rescued key HF features by reversing aberrant cytosolic and SR Ca^{2+} handling, restoration of a positive force–frequency-relationship and improved energetic biomarkers. Overall, successful validation of S100A1's therapeutic potential in a near-human in vivo and a human in vitro HF model clearly encourages further development towards clinical implementation. Moreover, these results readily motivate extended bi-directional research linking advanced understanding of S100A1's molecular framework to therapeutic and potentially adverse effects in a pre-clinical setting.

Overall, our current understanding of molecular HF mechanisms renders S100A1 a favorable HF gene therapy candidate for two major reasons: 1) S100A1 appears to control a Ca^{2+}-driven functional network, comprising the SR, myofilaments and mitochondria, that critically impacts cardiomyocyte contractile performance, excitability and energy homeostasis, further extending into growth and survival. Targeting defective S100A1 expression therefore represents not a "one target fits all" approach but multimodally enables advantageous restoration of several defective key systems involved in HF pathogenesis. 2) Abnormal S100A1 expression constitutes a chronic molecular defect amenable to long-term restoration by available state-of-the-art cardiac-targeted AAV-mediated S100A1 gene delivery under control of a constitutively active cardiomyocyte-specific promoter. Hence, by nature of the underlying molecular defect, a gene-based therapeutic strategy delivering the human S100A1 gene to dysfunctional cardiomyocytes in vivo appears as the most suitable means to revoke a chronic molecular key abnormality that drives HF progression.

5.2. "Second-generation" S100A1 heart failure gene therapy — a perspective on future needs and developments

However, to fully exploit its therapeutic potential, S100A1 HF gene therapy requires further development beyond current mechanistic understanding and state-of-the-art vector technology.

First, with respect to currently available AAV technology, patient's individuality regarding immunity and plasticity of expressed AAV co-receptors might guide development of "personalized" vectors. AAV-mediated cytotoxicity, as an example, might limit an increase in gene dosage when needed to achieve therapeutic effects in target cells and organs. In addition, density and ligand affinity of AAV-isoform biased co-receptors on target cells and organs such as epidermal growth factor receptor (EGFR) that facilitates internalization of AAV6 [49] contributes to gene load in target cells for a given amount of therapeutic vector. Hence, assaying patient's individual T-cell based cytotoxicity might guide development of low immunogenic AAVs tailored to patient's major histocompatibility (HLA) characteristics. Consequently, patient populations with similar HLA characteristics could be assigned to certain types of genetically engineered AAVs, in turn enabling therapeutically required high gene dosages with low immunogenicity. Additionally, positron emission tomography (PET)-based screening of patients for EGFR density and ligand affinity might guide therapeutic decisions on AAV6 dosages necessary to achieve the desired effects, thereby preventing over- and underdosage in different patient populations subjected to the same viral load.

Second, biological effects of defined S100A1 gene dosages are still difficult to predict as transgene expression results from a complex interplay between gene dosage, myocardial transduction efficiency, promoter activity and its decay within cardiomyocytes. Hence, it is mandatory to determine S100A1's gene dose-dependency in future studies to assess its therapeutic and toxic threshold in human-relevant HF models. To date, experimental therapeutic studies employing S100A1 gene transfer characterized a 2–4 fold increase in diseased myocardium thereby restoring normal to 1.5–2 fold S100A1 (over)expression without signs of acute or long-term toxicity. However, dose-escalation studies are needed to assess upper limits of S100A1 therapeutic actions either due to unspecific protein accumulation or intracellular "off-target" effects.

Third, standardized HF animal models do not adequately reflect heterogeneity of pathological plasticity of S100A1 expression found among HF patients at different stages of the disease. Among other aspects, standardized HF models do not accurately account for modifying factors such as concurrent pharmacological therapy; metoprolol treatment of HF rats, for instance, partially restored abnormal S100A1 expression apparently reducing the S100A1 gene dosage required to obtain a therapeutic effect. Thus, individual myocardial S100A1 expression levels and modifying factors need to be assessed prior to treatment in order to adequately adjust therapeutic S100A1 gene dosage application and avoid under- or overdosing.

Fourth, as severity of the disease fluctuates and adverse effects of gene-based treatment might occur, S100A1 transgene expression potentially requires both acute as well as chronic adjustments during the course of therapy. However, state-of-the-art gene therapy nowadays does not provide us with the opportunity to regulate or terminate transgene expression once implemented. Conclusively, controllable transgene vector technology endowed with an easy to

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use "kill-switch" is needed to adjust and terminate S100A1 transgene expression.

Fifth, even advanced AAVs still lack sufficient cardiac-specificity requiring invasive techniques such as retrograde venous perfusion in combination with cardiomyocyte-biased promoters to ensure cardiac-targeted expression of the therapeutic gene. Pre-existing immunity against AAV is another shortcoming that needs to be sufficiently addressed. However, as the field advances, it is expected that engineered AAVs with enhanced cardiac tropism will be developed and made available for clinical use in order to circumvent the need of invasive delivery procedures. In addition, as the anticipated therapeutic benefit becomes evident in clinical trials, more elaborated interventions such as plasmapheresis might be applicable, enabling "one-time" AAV-mediated gene therapy in patients with pre-existing antibodies.

Conclusively, for a "second-generation" S100A1 HF gene therapy to finally emerge as a viable clinical therapeutic option, we need to embark on the current momentum due to ongoing "first-generation" clinical phase I/II trails targeting abnormal Ca^{2+} cycling in HF. At the same time, however, we need to foster development towards controllable and personalized S100A1 gene-based HF treatment employing a molecular target with a favorable and unique therapeutic profile.

Acknowledgments

This study was supported by grants of the National Institute of Health (RO1 HL92130 and RO1 HL92130-02 S1 to PM), Deutsche Forschungsgemeinschaft (562/1-1 to PM) and Bundesministerium fuer Bildung und Forschung (01GU0572 to PM).

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