

Cardiac S100A1 Protein Levels Determine Contractile Performance and Propensity Toward Heart Failure After Myocardial Infarction

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Background—Diminished cardiac S100A1 protein levels are characteristic of ischemic and dilated human cardiomyopathy. Because S100A1 has recently been identified as a Ca^{2+} -dependent inotropic factor in the heart, this study sought to explore the pathophysiological relevance of S100A1 levels in development and progression of postschemic heart failure (HF).

Methods and Results—S100A1-transgenic (STG) and S100A1-knockout (SKO) mice were subjected to myocardial infarction (MI) by surgical left anterior descending coronary artery ligation, and survival, cardiac function, and remodeling were compared with nontransgenic littermate control (NLC) and wild-type (WT) animals up to 4 weeks. Although MI size was similar in all groups, infarcted S100A1-deficient hearts (SKO-MI) responded with acute contractile decompensation and accelerated transition to HF, rapid onset of cardiac remodeling with augmented apoptosis, and excessive mortality. NLC/WT-MI mice, displaying a progressive decrease in cardiac S100A1 expression, showed a later onset of cardiac remodeling and progression to HF. Infarcted S100A1-overexpressing hearts (STG-MI), however, showed preserved global contractile performance, abrogated apoptosis, and prevention from cardiac hypertrophy and HF with superior survival compared with NLC/WT-MI and SKO-MI. Both Gq-protein-dependent signaling and protein kinase C activation resulted in decreased cardiac S100A1 mRNA and protein levels, whereas Gs-protein-related signaling exerted opposite effects on cardiac S100A1 abundance. Mechanistically, sarcoplasmic reticulum Ca^{2+} cycling and β -adrenergic signaling were severely impaired in SKO-MI myocardium but preserved in STG-MI.

Conclusions—Our novel proof-of-concept study provides evidence that downregulation of S100A1 protein critically contributes to contractile dysfunction of the diseased heart, which is potentially responsible for driving the progressive downhill clinical course of patients with HF. (*Circulation*. 2006;114:1258-1268.)

Key Words: calcium ■ contractility ■ heart failure ■ myocardial infarction

S100A1, a dimeric Ca^{2+} -binding protein of the EF-hand type, belongs to the S100 protein family in which members are predominantly expressed in a tissue-specific manner.^{1,2} S100A1, highly prevalent in cardiac and less abundant in skeletal muscle, shows a subcellular location at the sarcoplasmic reticulum (SR), myofilaments, and mitochondria.³⁻⁷ Like other S100 proteins, S100A1 seems to require a Ca^{2+} -dependent conformational change to recognize downstream effectors, thereby eliciting a specific biological response as a Ca^{2+} -sensor protein in the heart.⁸ We previously identified S100A1 as a crucial regulator of cardiac and skeletal muscle contractile performance exerting a marked positive inotropic effect in vitro and in vivo.^{5-7,9-11} In particular, S100A1 increases contractile performance of cardiac and skel-

etal muscle by enhancing intracellular Ca^{2+} transients through increased excitation-contraction coupling gain.

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This phenotypic change in cardiac muscle is mediated exclusively through improved SR Ca^{2+} cycling because S100A1 affects neither L-type Ca^{2+} channel nor sodium-calcium exchanger activity.^{5,6,9-11} Mechanistically, S100A1 interacts both with the SR Ca^{2+} -release channel (RyR2) and the SR Ca^{2+} pump (SERCA2) in a Ca^{2+} -dependent manner, resulting in enhanced Ca^{2+} -induced SR Ca^{2+} release and SR Ca^{2+} reuptake, respectively.^{6,9-11} In addition, S100A1 can decrease the diastolic SR Ca^{2+} leak through attenuated Ca^{2+} -spark frequency, suggesting a biphasic Ca^{2+} -dependent mod-

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ulation of the RyR2 through interaction with S100A1.¹² In addition to regulating SR function, S100A1 has been shown to modulate Ca²⁺ responsiveness and passive tension development of cardiac myofilaments via a Ca²⁺-dependent interaction with cardiac titin.^{10,13}

In terms of clinical application, the therapeutic potential of S100A1 has become apparent because failing human myocardium displays a marked loss of S100A1 expression at both the transcriptional and translational levels.¹⁴ However, signaling pathways involved in S100A1 downregulation have not yet been elucidated. Recently, we provided the first evidence for the therapeutic effectiveness of viral-based S100A1 gene transfer in an experimental heart failure (HF) model.⁶ Intracoronary adenoviral S100A1 gene delivery restored diminished S100A1 protein levels and dysfunctional SR Ca²⁺ cycling that, in turn, rescued contractile function and reversed remodeling of failing myocardium.⁶ However, the favorable therapeutic effect of cardiac S100A1 gene delivery is not confined to the chronically failing heart but is also effective in a setting of acute myocardial infarction (MI), in which intracoronary adenoviral S100A1 gene delivery prevented acute post-MI contractile dysfunction and remodeling in experimental MI as a result of the preservation of intracellular Ca²⁺ homeostasis.¹⁵ These studies demonstrate S100A1 gene delivery as a potential clinically powerful and novel therapeutic strategy to treat both the acute and chronically failing heart.

Despite the loss of S100A1 expression in end-stage failing human hearts,¹⁴ the pathophysiological contribution of decreased cardiac S100A1 expression for the development and progression of HF per se has not yet been examined. In the present study, we addressed this issue in a clinically relevant setting. S100A1-transgenic (STG) and S100A1-knockout (SKO) mice displaying a cardiac-specific S100A1 protein overexpression and deficiency, respectively,^{11,16} were subjected to MI, and the course of post-MI remodeling, cardiac failure, and molecular alterations was comprehensively studied *in vitro* and *in vivo*. We found a significant disparity in the post-MI phenotype in SKO versus STG mice. Our results clearly show that levels of cardiac S100A1 play a critical role in post-MI HF and provide novel evidence that downregulation of this Ca²⁺ sensor aggravates contractile dysfunction of the compromised heart and potentially contributes to the steady downhill clinical course in HF patients. However, prevention of this S100A1 loss confers protection from post-MI remodeling and HF.

Methods

The generation of the SKO and STG mouse lines was previously described.^{11,16} The experimental procedures for left coronary artery ligation, assessment of infarct size and survival, and echocardiographic and hemodynamic analysis of cardiac function are described in the expanded Methods section in the online-only Data Supplement. Detailed procedures for assessment of myocardial histopathology and apoptosis, β -adrenergic receptor density and adenylyl cyclase activity, calcium measurements, quantitative real-time polymerase chain reaction, Western blot analysis, and isolation and culture of rat neonatal ventricular cardiomyocytes (NVCMs) are available in the expanded online-only Data Supplement. Data are generally expressed as mean \pm SEM. An unpaired 2-tailed Student *t* test and 2-way repeated ANOVA were performed for between-group

comparisons. Survival analysis was performed by the Kaplan-Meier method, and between-group differences in survival were tested by the log-rank test. For all tests, a probability value <0.05 was considered significant. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

S100A1 Improves Survival After MI

Nontransgenic littermate control (NLC) and wild-type (WT) mice were subjected to left anterior descending artery ligation (NLC/WT-MI) (Figure I in the online-only Data Supplement), resulting in $\approx 37\%$ infarcted left ventricle (LV), which was not significantly different from infarcted STG (STG-MI) and SKO (SKO-MI) mice (Figure 1A and 1B). Despite similar infarct size, 4-week post-MI mortality in SKO-MI (82%) was significantly higher than that in NLC/WT-MI (57%) (Figure 1C). In contrast, STG-MI (33%) displayed significantly superior survival compared with NLC/WT-MI and SKO-MI mice (Figure 1C). There were no deaths in the NLC/WT, STG, and SKO sham groups (data not shown). An extended description of post-MI survival characteristics is available in the online-only Data Supplement.

S100A1 Prevents Development of Postischemic Heart Failure

Serial echocardiography and LV catheterization were performed to assess the contribution of different cardiac S100A1 protein levels on contractile performance after MI. Figure 2A and 2B provide representative original tracings of M-mode echocardiography and invasive hemodynamics 7 days after MI in all groups compared with sham NLC/WT. Ischemic injury provoked an acute and progressive decrease of systolic contractile function in NLC/WT-MI hearts, as evidenced by impaired fractional shortening (Figure 2C) and dP/dt_{max} (Figure 2D). Corresponding alterations were found for dP/dt_{min} (data not shown) and LV end-diastolic pressure (LVEDP) (Figure 2E). Concomitantly, expression levels of S100A1 protein in remote NLC/WT-MI myocardium continuously declined to 35% of levels found in sham hearts (Figure 2F and 2G) (0.07 ± 0.01 versus 0.21 ± 0.02 ng S100A1 protein/ μ g myocardial protein; $P < 0.05$; $n = 6$; Figure II in the online-only Data Supplement). In contrast, STG-MI hearts displaying persistently elevated S100A1 protein levels (0.72 ± 0.03 ng S100A1 protein/ μ g myocardial protein) (Figure 2F and 2G and Figure II in the online-only Data Supplement) exerted only an acute reduction in contractile performance in response to MI, with further preservation of systolic and diastolic cardiac function at the level of sham-NLC/WT hearts (Figure 2C to 2E). However, SKO-MI hearts, lacking S100A1 protein (Figure 2F), responded with acute cardiac decompensation and accelerated transition to HF (Figure 2C to 2E) during the study period.

Gq-Protein–Coupled Receptor Agonists Involving Protein Kinase C Downregulate Cardiac S100A1 mRNA and Protein Expression

To delineate pathways causing loss of myocardial S100A1 expression in the course of post-MI HF, NVCMs were subjected to prohypertrophic Gq-protein–coupled receptor

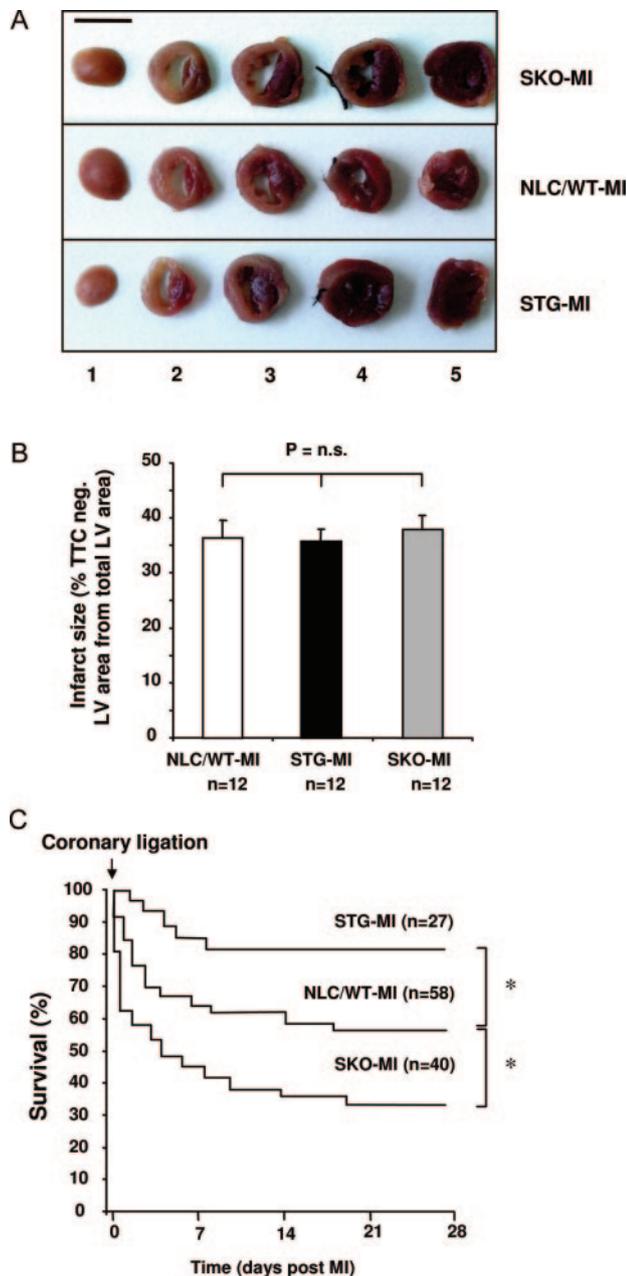


Figure 1. Impact of different cardiac S100A1 protein levels on infarct size and posts ischemic survival. **A**, Representative images of sectioned 2-3-5-triphenyl tetrazolium chloride (TTC)-stained SKO-MI, NLC/WT-MI, and STG-MI hearts 24 hours after surgery. Apical to basal direction is indicated by numbers 1 to 5. Nons ischemic myocardium in slices 4 and 5 exhibits a deep red color typical for positive TTC staining. Note the progressive fainting in slice 3 to 1 below the blue suture (slice 4) reflecting ischemic myocardium. Bar=1 cm. **B**, Results of morphometric quantification of percentage of infarcted area 24 hours after coronary ligation in NLC/WT-MI, STG-MI, and SKO-MI mice. **C**, Kaplan-Meier survival analysis. Percentages of surviving STG-MI, NLC/WT-MI, and SKO-MI mice were plotted. **P*<0.05 vs NLC/WT-MI.

agonists such as endothelin-1 (ET-1) and phenylephrine. As previously described, both Gq-protein-coupled receptor agonists exerted a significant time- and dose-dependent upregulation and downregulation of *ANP* and *SERCA2a* mRNA levels, respectively (data not shown). Stimulation of NVCMs

with ET-1 and phenylephrine resulted in a dose- and time-dependent decrease of *S100A1* mRNA levels to 30% to 40% compared with levels seen in control cells (Figure 3A, 3B, 3D, and 3E). These transcriptional alterations were translated into a significant ≈ 2 -fold reduction of S100A1 protein levels compared with untreated NVCMs (Figure 3C and 3F). Because protein kinase C (PKC) is known to play a crucial role in Gq-protein-coupled receptor-dependent hypertrophic signaling, NVCMs were subjected to phorbol 12-myristate 13-acetate (PMA) to assess the impact of activated PKC on S100A1 transcription and translation. PMA caused a significantly stronger dose- and time-dependent decline in *S100A1* mRNA expression than Gq-protein-coupled receptor agonists (Figure 3G and 3H) that was translated into a significant ≈ 2 -fold reduction of S100A1 protein levels (Figure 3I).

Gs-Protein-Dependent Signaling Involving cAMP Stimulates Cardiac S100A1 mRNA and Protein Expression

In contrast, Gs-dependent signaling through the β -adrenergic receptor cascade via isoproterenol and forskolin resulted in significantly enhanced S100A1 mRNA and protein levels in NVCMs (Figure III in the online-only Data Supplement), suggesting both negative and positive regulatory feedback loops via Gq- and Gs-dependent signaling, respectively. An extended description of this finding is given in the online-only Data Supplement.

S100A1 Inhibits Posts ischemic LV Remodeling and Abrogates Apoptosis

NLC/WT-MI hearts developed robust LV hypertrophy 28 days after MI, as indicated by reactivation of fetal and extracellular matrix gene expression (Figure 4A to 4F), increased ratio of heart to body weight (Figure 5A), enlarged cardiomyocyte cross-sectional area (Figure 5B), and LV end-diastolic diameter (LVEDD) (Figure 5C). Enhanced cardiac S100A1 expression prevented LV remodeling in STG-MI hearts, evidenced by significant suppression of fetal gene expression (Figure 4A to 4F), nearly unchanged ratio of heart to body weight (Figure 5A), and cardiomyocyte cross-sectional area (Figure 5B) as well as maintained LV geometry (Figure 5C) compared with sham hearts. Absence of cardiac S100A1 protein, however, clearly accelerated LV remodeling in SKO-MI hearts displaying a premature onset of fetal and extracellular matrix gene expression (Figure 4A to 4F) that was accompanied by increased ratio of heart to body weight (Figure 5A) and cardiomyocyte cross-sectional area (Figure 5B) as well as enlarged LVEDD 7 days after MI (Figure 5C).

A similar polarized pattern was found for myocyte loss through apoptosis. Significantly more terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive nuclei were prevalent in SKO-MI remote myocardium, indicating a higher incidence of apoptosis in the absence of S100A1 (Figure 5D). Accordingly, analysis of caspase 3 activity in myocardial tissue revealed a significantly higher activity in SKO-MI remote myocardium (Figure IV in the online-only Data Supplement). In contrast, STG-MI hearts displayed a significantly lower rate of TUNEL-positive nuclei and caspase 3 activity compared with SKO-MI and

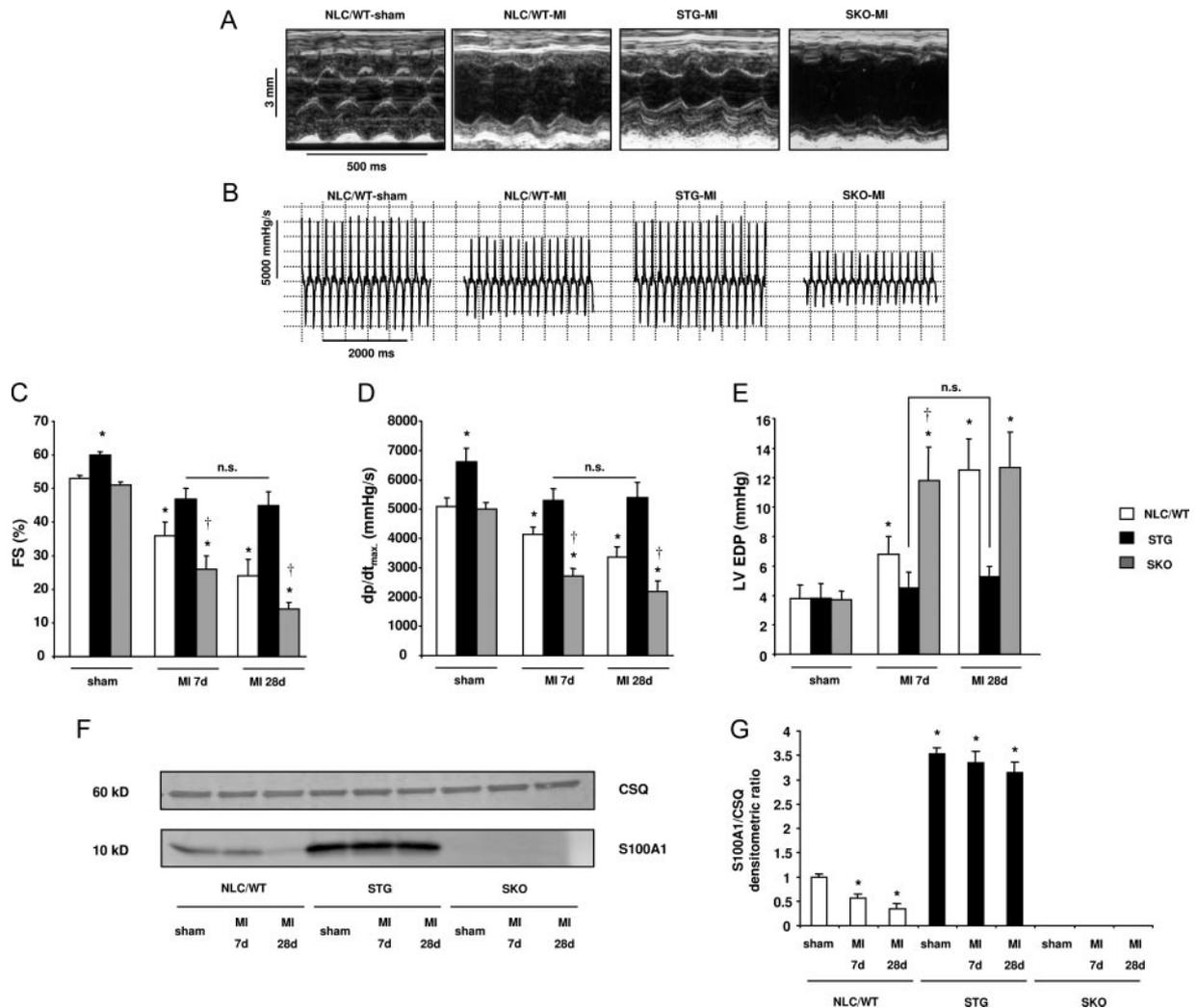


Figure 2. Influence of different cardiac S100A1 protein abundance on postischemic hemodynamic function and transition to HF. A and B, Representative M-mode echocardiography and original tracings of maximal and minimal first derivative of LV pressure rise and fall taken 7 days after MI in sham-NLC/WT, NLC/WT-MI, STG-MI, and SKO-MI mice. C through E, Quantitative analysis of fractional shortening (FS), maximal first derivative of LV pressure rise (dp/dt_{max}), and LVEDP (mm Hg) in sham and infarcted mice assessed 7 and 28 days after MI. Hemodynamics in sham mice were not different 7 or 28 days after surgery and are shown for the latter group. Mean heart rate (503±22 bpm) in response to our anesthesia protocol was similar in all groups (data not shown). F and G, A representative serial S100A1 Western blot and densitometric quantitative analysis of the S100A1/CSQ ratio in sham and infarcted NLC/WT, STG, and SKO mice. S100A1 protein was not detectable in SKO mice. Unchanged CSQ protein abundance was used for normalization, and densitometric values are given as arbitrary units. *P<0.05 vs sham NLC/WT; †P<0.05 vs NLC/WT-MI; n=8 in each group.

NLC/WT-MI hearts (Figure 5D and Figure IV in the online-only Data Supplement). Finally, we investigated the activation of mitogen-activated protein kinase (MAPK; p44/42) and stress-activated protein kinase (SAPK; p38, p54/46) signaling modules in STG-MI hearts. Compared with NLC/WT-MI, phosphorylation status of p44/42, p38 as well as p54/46 was blunted in STG-MI remote myocardium (Figure 5E and 5F). In contrast, significantly augmented phosphorylation of both MAPK and SAPK was evident in SKO-MI myocardium compared with both NLC/WT-MI and STG-MI (Figure 5E and 5F).

S100A1 Inhibits Postischemic β-Adrenergic Signaling Defects

SKO-MI hearts displayed an accelerated decrease in β-adrenergic receptor density (Figure 6A) and elevated

GRK2 protein levels 7 days after MI (Figure 6B) compared with NLC/WT-MI hearts that showed similar alterations 28 days after MI (Figure 6A and 6B). Impaired cAMP formation in response to isoproterenol confirmed severe uncoupling of β-adrenergic receptor signaling in remodeled SKO-MI hearts (Figure 6C). In contrast, remote STG-MI myocardium neither exerted a significant suppression of β-adrenergic receptor density (Figure 6A) nor increased GRK2 levels (Figure 6B) during the study period, and this was associated with normal cAMP production (Figure 6C). In accord with accelerated in vitro alterations of β-adrenergic receptor signaling, SKO-MI hearts showed impaired in vivo contractile β-adrenergic receptor responsiveness (Figure 6D). Moreover, the phosphorylation state of phospholamban at ser-16 7 days after MI was impaired in SKO-MI hearts (Figure 7A). Similar but less pronounced alterations were evident in NLC/WT-MI hearts (Figures 6D and 7A).

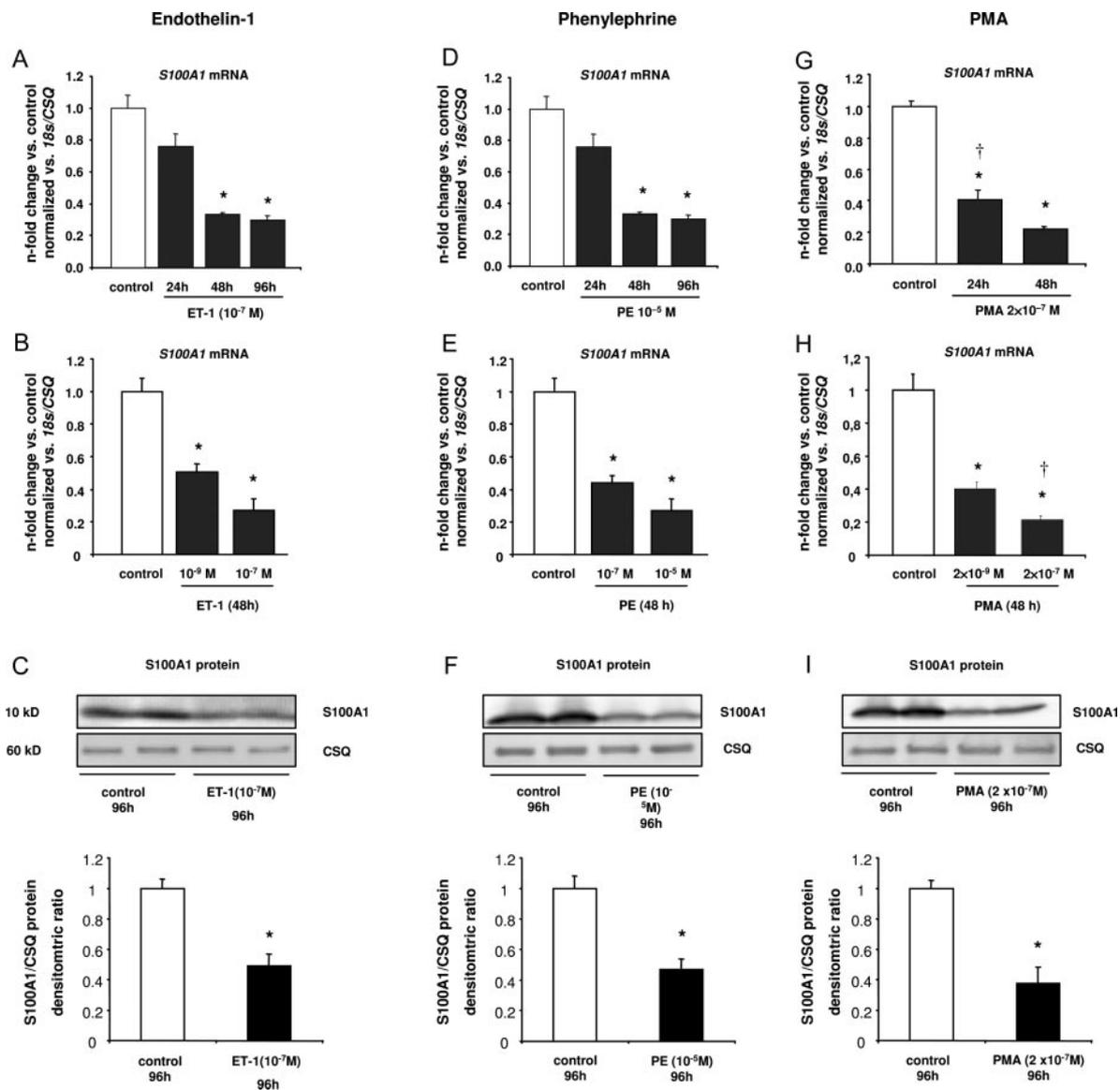


Figure 3. Effect of Gq-protein-coupled receptor agonists and activated PKC on cardiac S100A1 expression in vitro. A/B, D/E, and G/H, Time- and dose-dependent decrease of cardiac S100A1 mRNA levels in NVCMs in response to Gq-coupled receptor agonists ET-1 and phenylephrine as well as to the PKC activator PMA. C, F, and I, Decline of cardiac S100A1 protein levels in NVCMs due to the stimulation with ET-1, phenylephrine (PE), and PMA. Unchanged 18s and CSQ mRNA and CSQ protein abundance were used for mRNA and protein normalization, respectively. Densitometric values are given as arbitrary units and normalized to control. n=3 different NVCM preparations. * $P < 0.05$ vs control; † $P < 0.05$ vs corresponding ET-1 and phenylephrine stimulation.

In contrast, STG-MI hearts exerted a robust in vivo responsiveness to the β -adrenergic receptor agonist isoproterenol (Figure 6D), accompanied by a normal-like increase in phospholamban ser-16 phosphorylation (Figure 7A).

S100A1 Preserves SR Ca²⁺ Cycling After MI

SERCA2 protein levels were found to be significantly decreased 4 weeks after MI in failing NLC/WT-MI hearts (Figure 7B). Importantly, accelerated transition to HF was associated with an earlier decline of SERCA2 expression in SKO-MI hearts (Figure 7B). STG-MI remote myocardium, however, showed preserved SERCA2 expression up to 4 weeks after MI (Figure 7B). Like decreased abundance, enhanced nitration of SERCA2a has recently been reported to play a role in SR function and hence contractile performance

in failing human myocardium.¹⁷ We found a significantly higher level of SERCA2 nitration in SKO-MI than NLC/WT-MI compared with sham NLC/WT and SKO myocardium 28 days after MI (Figure 7C). In contrast, STG-MI myocardium showed only a small increase in SERCA2 nitration (Figure 7C).

To investigate the functional consequences of altered SERCA2 abundance and nitration, we assessed Ca²⁺ uptake in SR microsomes derived from remote myocardium of infarcted and sham hearts. STG hearts displayed a significantly higher Ca²⁺ uptake than NLC/WT and SKO, and this gain in function was nearly preserved in STG-MI SR microsomes (Figure 8A and 8B). In contrast, SR vesicles from failing NLC/WT-MI exerted a significant reduction in Ca²⁺ uptake that was more pronounced in failing SKO-MI (Figure

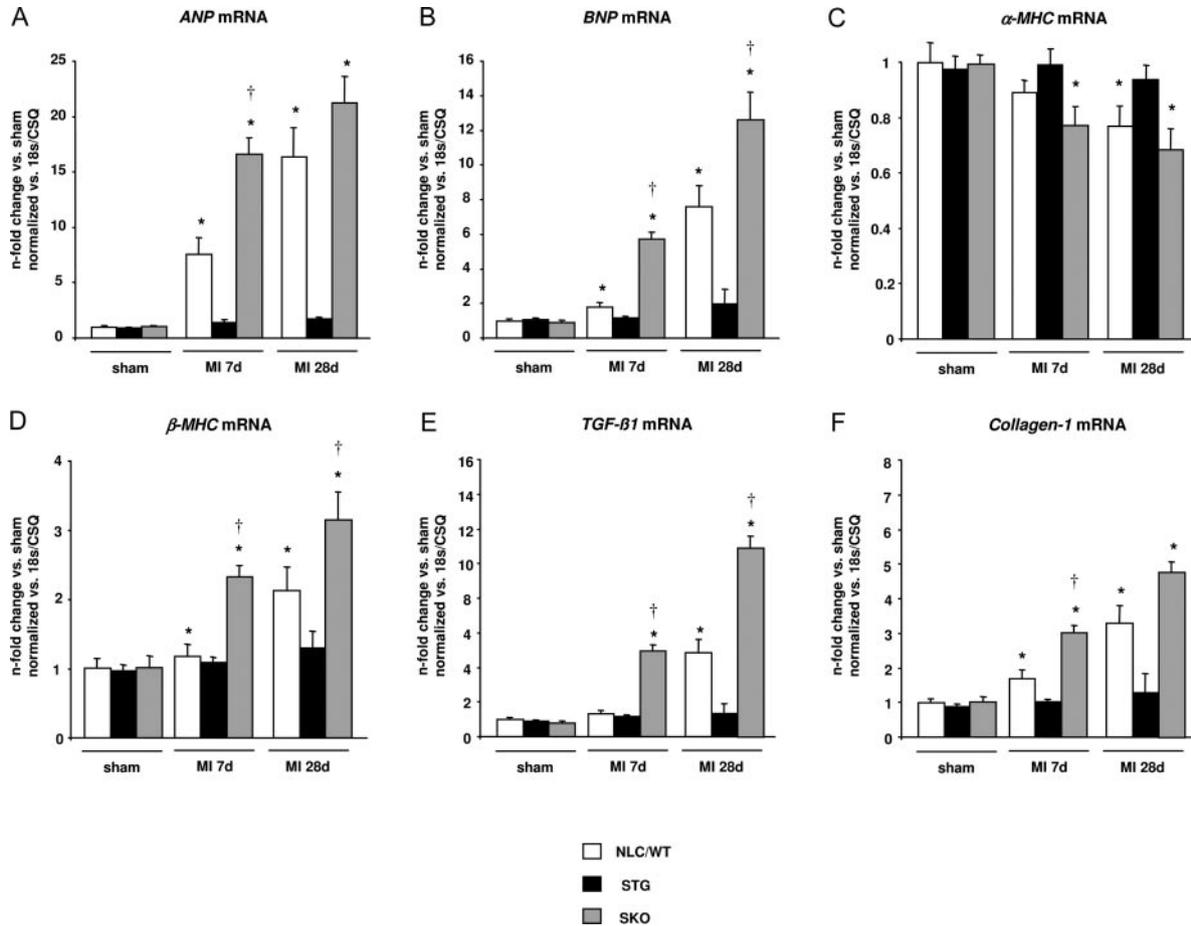


Figure 4. Impact of cardiac S100A1 protein levels on postischemic reactivation of fetal and extracellular matrix gene expression. A through F, Quantitative analysis of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), α -myosin heavy chain (α -MHC), β -myosin heavy chain (β -MHC), transforming growth factor- β 1 (TGF- β 1), and collagen-1 mRNA expression in remote myocardium from sham and infarcted hearts 7 and 28 days after MI. Unchanged 18s rRNA and CSQ mRNA were used for normalization. * $P < 0.05$ vs sham NLC/WT; † $P < 0.05$ vs NLC/WT-MI; n=5 in each group.

8A and 8B). Subsequent analysis of SR Ca^{2+} leak revealed a significant increase in Ca^{2+} leakage in SR microsomes derived from failing NLC/WT-MI, and this defect was significantly more pronounced in SKO-MI myocardium (Figure 8C and 8D). Likewise, in Ca^{2+} uptake, the gain in caffeine-induced SR Ca^{2+} release was nearly preserved in SR vesicles from STG-MI (Figure 8E and 8F). However, the decrease of SR Ca^{2+} release was more pronounced in failing SKO-MI than in NLC/WT-MI myocardium (Figure 8E and 8F).

Discussion

The phenotype of S100A1-transgenic and S100A1-null mice reported here demonstrates that S100A1, although dispensable for cardiac muscle development and function under basal conditions,¹⁶ is a critical factor required for the compromised heart to functionally adapt to increased mechanical workload under pathophysiological conditions. Notably, the absence of S100A1 significantly accelerates the development of contractile dysfunction after MI with a rapid onset of cardiac remodeling and transition to HF combined with excessive mortality. Conversely, increased cardiac S100A1 expression effectively preserves global contractile performance even in the face of a significant LV infarct and protects the heart from

maladaptive hypertrophy and HF with superior survival. Thus, our study clearly provides proof of principle for the essential pathophysiological relevance of altered cardiac S100A1 expression in the course of HF per se.

Because we have previously shown that S100A1 evokes a Ca^{2+} -dependent positive inotropic pathway,^{5,6,9–11,15} our data suggest that the hypercontractile remote myocardium in STG-MI hearts can functionally compensate for the loss of viable myocardium, thereby enabling even the damaged heart to successfully cope with chronically elevated hemodynamic stress. Subsequently, a preserved normal-like systolic and diastolic contractile function of STG-MI hearts seems to mechanistically prevent injurious signaling from both biomechanical overload and maladaptive hyperadrenergic activation. That, in turn, apparently eliminates the most important triggers causing cardiac remodeling.¹⁸ This conclusion is further corroborated by the finding that STG-MI remote myocardium exhibited neither relevant molecular β -adrenergic receptor signaling defects nor impaired β -adrenergic receptor responsiveness in vivo and showed nearly preserved diastolic functional parameters, indicating unaltered cardiac preload. In accord with this salient feature, we have previously provided evidence for ameliorated

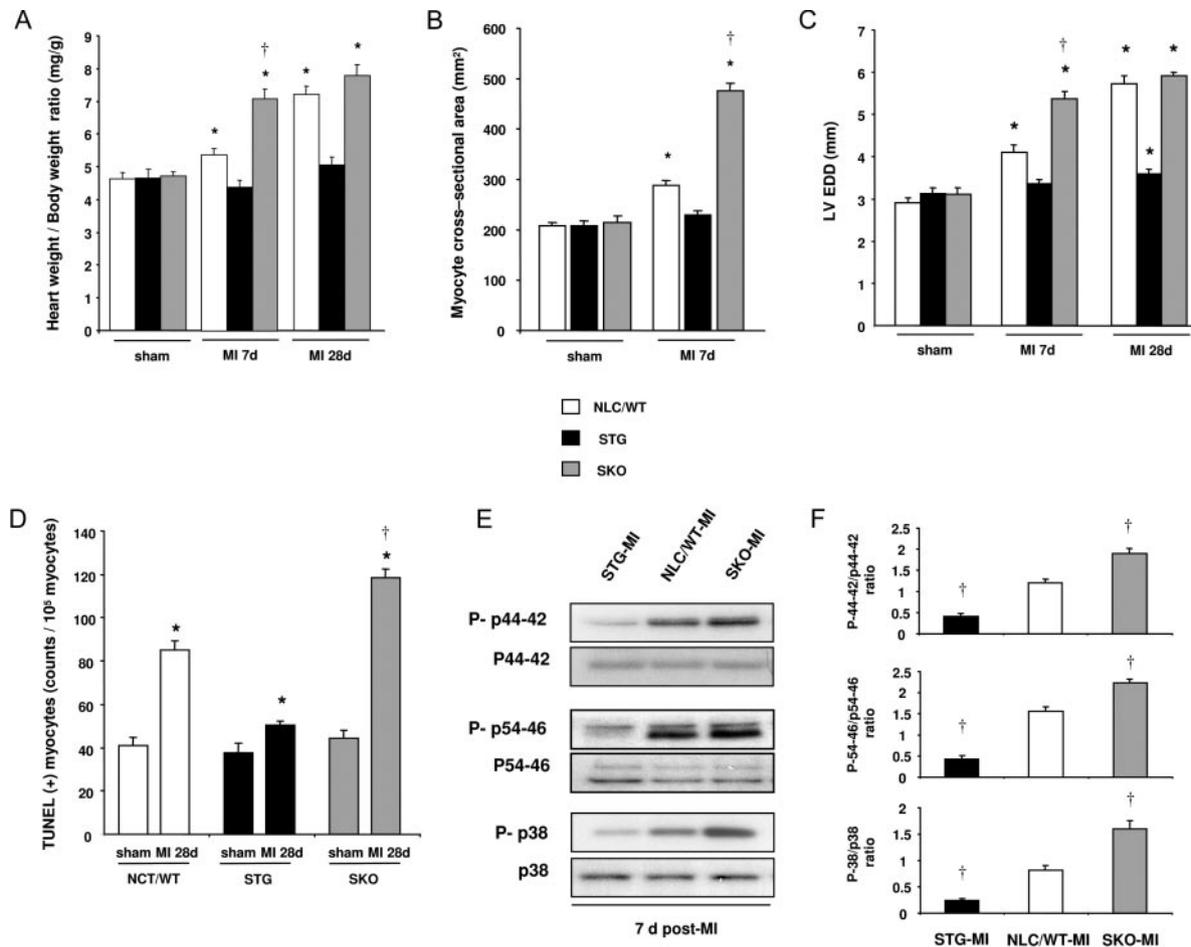


Figure 5. Influence of different cardiac S100A1 protein abundance on LV remodeling, apoptosis, and MAPK/SAPK signaling. A through C, Quantitative analysis of ratio of heart weight to body weight, cardiomyocyte cross-sectional area, and LVEDD after MI compared with sham-operated mice. D, Quantitative assessment of TUNEL-positive (+) cardiomyocytes in remote myocardium from sham and infarcted hearts 28 days after MI. E and F, Representative Western blots and quantitative estimation for the ratio of phosphorylated (P) and nonphosphorylated forms of p44-42, p54-46, and p38 in remote myocardium from STG-MI, NLC/WT-MI, and SKO-MI hearts 7 days after MI ($n=5$ in each group). Unchanged CSQ protein levels were used for normalization (data not shown). * $P<0.05$ vs sham NLC/WT; † $P<0.05$ vs NLC/WT-MI; $n=5$ in each group.

β -adrenergic receptor desensitization in failing and acutely infarcted myocardium through viral-based cardiac S100A1 gene therapy in vivo.^{6,15}

Although it is unknown whether S100A1 directly interferes with components of the hypertrophic signaling machinery, our study provides novel evidence for S100A1-mediated abrogation of GRK2 upregulation that might inhibit β -arrestin-dependent MAPK and SAPK prohypertrophic signaling in remote post-MI myocardium. Moreover, the finding that GRK2 upregulation is enhanced in the more rapidly deteriorating SKO-MI hearts is consistent with our previous findings that GRK2 is associated with early HF progression in other mouse models.^{19,20} This is in addition to a reversal of the fetal gene program, which has previously been observed with S100A1 overexpression in myocytes and is in agreement with previous reports showing reverse remodeling of failing and infarcted myocardium in vivo after adenoviral S100A1 gene transfer.^{6,15,21}

Similarly, apoptosis, thought to contribute to the progressive deterioration of contractile function through “dropout” of cardiomyocytes,²² was minimized in STG-MI remote myo-

cardium. Several previously reported mechanisms might contribute at least in part to this effect. First, extracellular S100A1 protein has recently been shown to exert direct antiapoptotic actions at cardiomyocytes in vitro.²³ Second, STG-MI remote myocardium exerts no induction of S100B protein expression (data not shown), which extracellular release from myocardium has recently been shown to exhibit potent proapoptotic actions on cardiomyocytes.²⁴ Finally, S100A1-mediated prevention of increased wall stress, commonly known as a potent trigger for apoptosis, might indirectly contribute to the prosurvival effects on myocardium.

In contrast to the favorable effects seen in STG-MI myocardium, lack of cardiac S100A1 expression immediately disabled the infarcted heart to functionally adapt to increased biomechanical stress, resulting in an accelerated onset of cardiac hypertrophy and reactivation of a fetal gene program. Importantly, these striking results are in agreement with a previous report showing immediate contractile failure but unaltered cardiac hypertrophy in SKO mice subjected to transaortic constriction.¹⁶ Similar alterations, albeit later,

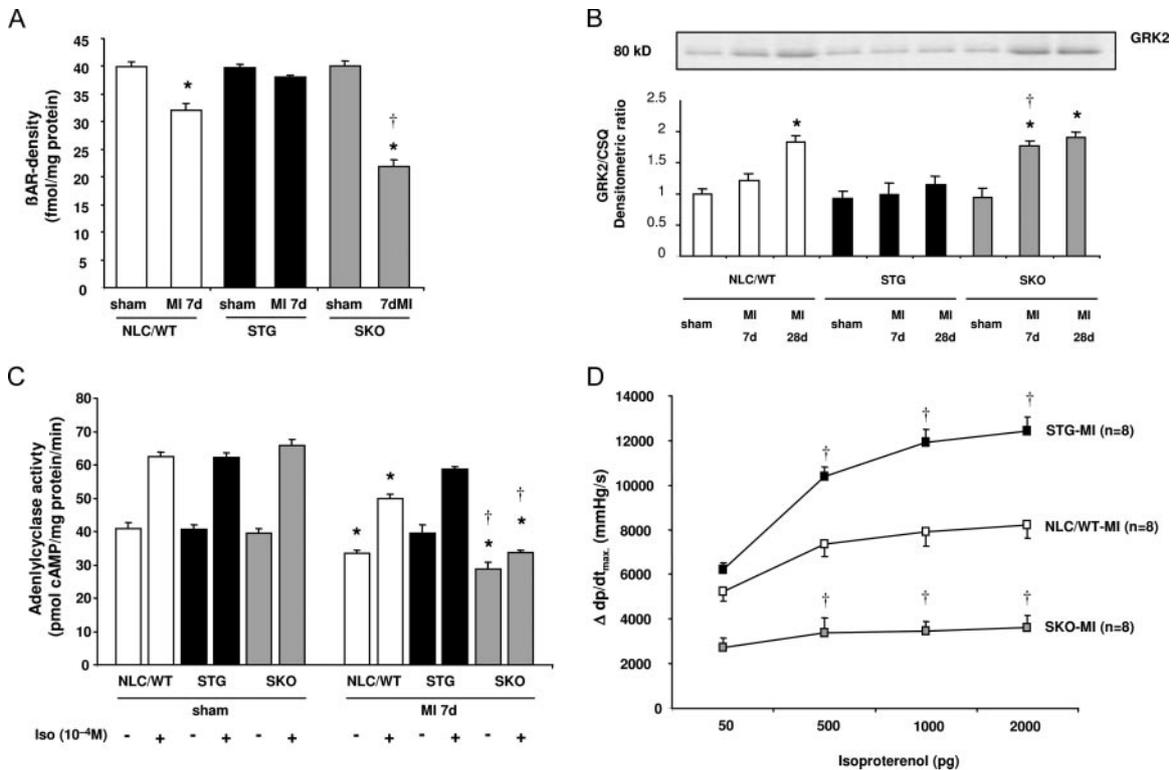


Figure 6. Effect of different cardiac S100A1 protein levels on posts ischemic β -adrenergic receptor (β AR) signaling. A, Cardiac β -adrenergic receptor density 7 days after MI in sham and infarcted NLC/WT, STG, and SKO hearts. B, Representative serial GRK2 Western blot and densitometric quantitative analysis of the GRK2/CSQ ratio in sham and infarcted NLC/WT, STG, and SKO mice. Densitometric values are given as arbitrary units (n=5 in each group). C, Adenylyl cyclase activity 7 days after MI in membrane fractions obtained from sham and infarcted hearts under basal conditions and in response to isoproterenol (Iso) (n=5 in each group). D, Inotropic reserve of NLC/WT-MI, STG-MI, and SKO-MI in response to β -adrenergic receptor stimulation 7 days after MI by the change in dp/dt_{max} to increasing dosages of isoproterenol (n=8 in each group). * $P < 0.05$ vs sham NLC/WT; † $P < 0.05$ vs NLC/WT-MI.

were observed in infarcted control mice that displayed a progressive downregulation of S100A1 protein even in the stage of LV hypertrophy. SKO-MI hearts, however, immediately evolved to overt contractile failure, indicating that even lower S100A1 levels seen in post-MI NLC hearts can be beneficial compared with a lack of expression.

Because SKO mice have normal cardiac function at rest but impaired cardiac reserve in response to both β -adrenergic receptor stimulation and incremental extracellular Ca^{2+} concentration,¹⁶ this finding strongly supports the notion of a fundamental downstream defect in SKO hearts. Accordingly, isolated ventricular cardiomyocytes derived from control SKO hearts displayed nearly normal Ca^{2+} cycling under basal conditions but blunted cytosolic Ca^{2+} transients in response to enhanced transsarcolemmal Ca^{2+} influx and β -adrenergic receptor stimulation in vitro (data not shown). Thus, the defective in vivo “flight-or-fight” response characteristic for to SKO myocardium might apparently rely on inadequate mobilization of intracellular Ca^{2+} cycling disabling SKO-MI from the first to adequately compensate for the loss of myocardium even in response to concentrated β -adrenergic receptor stimulation. In turn, absence of S100A1 promoted an earlier onset of β -adrenergic receptor dysfunction than in infarcted control mice that had residual S100A1 expression. Hence, the immediate failure of functional compensation in STG-MI hearts resulted in accelerated hyperadrenergic acti-

vation and excessive mechanical overload, promoting the seamless transition from hypertrophy to cardiac dilation and failure.

In accord with clinical results, the accelerated kinetics of this sequence in SKO-MI mice resulted in excessive mortality, confirming cardiac hypertrophy as a major predictor for HF and sudden death.²² In contrast, favorable hemodynamic effects in STG-MI were translated into superior survival. This is important to note because other strategies targeting SR Ca^{2+} cycling, eg, by means of SERCA2 gene addition, can result in impaired survival in response to ischemic cardiac injury.²⁵ Thus, under clinical conditions, application of a therapeutic target like S100A1 that seems to modulate cardiac excitation-contraction coupling through >1 molecular mechanism might be a superior approach even in response to repetitive ischemic periods.

Because altered intracellular Ca^{2+} cycling plays a key role in the development of HF,²⁶ we analyzed both abundance and function of major SR Ca^{2+} cycling proteins as a surrogate for altered cardiac intracellular Ca^{2+} handling. STG-MI remote myocardium displayed preserved supranormal SR function that might account in part for the prolonged resistance to increased hemodynamic workload. These results are supported by our previous data showing rescued intracellular Ca^{2+} turnover and SR Ca^{2+} handling in failing myocardium through intracoronary S100A1 adenoviral gene transfer.⁶

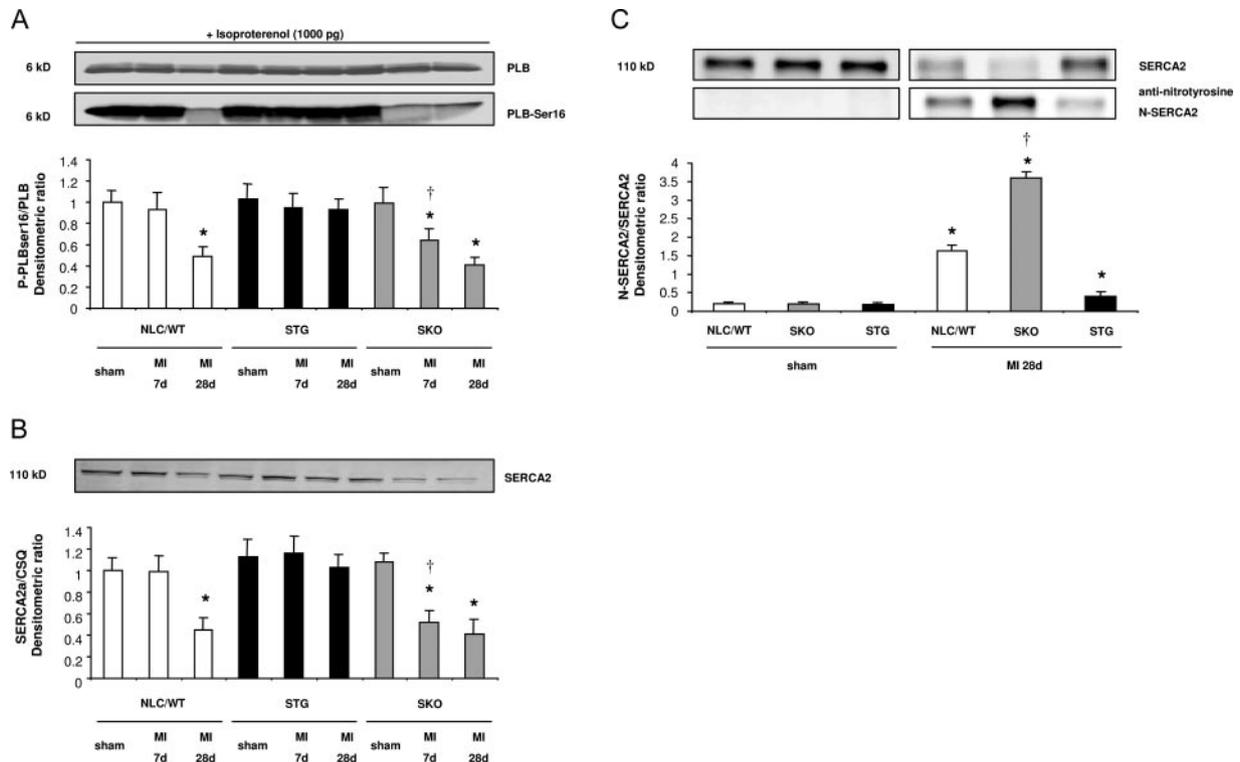


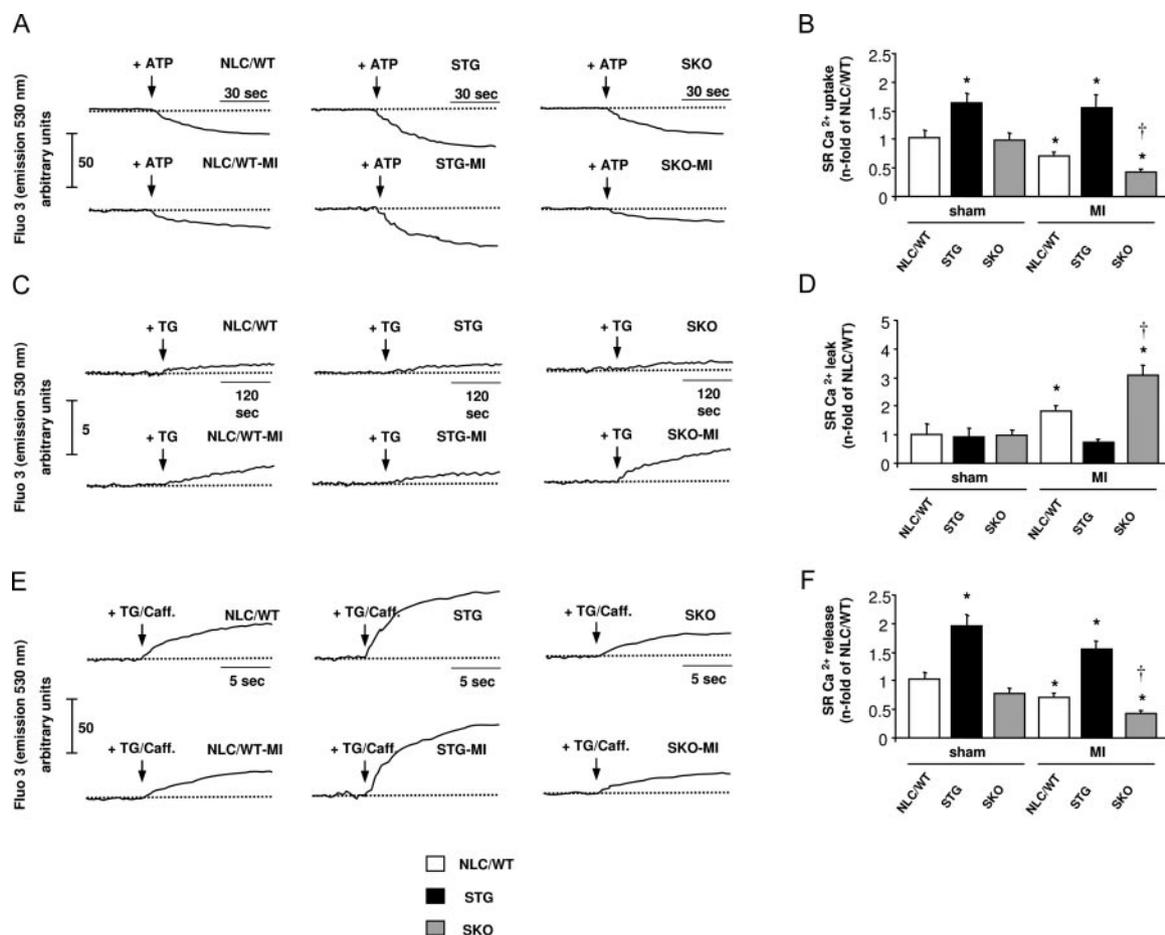
Figure 7. Impact of different cardiac S100A1 protein abundance on phospholamban (PLB)-serine 16 (ser16) phosphorylation, SERCA2 expression levels, and posttranslational modification. A through C, A representative Western blot and quantitative analysis of the phosphorylated phospholamban (P-PLB)-serine 16/phospholamban ratio, SERCA2a expression, and relative levels of nitrotyrosylation of immunoprecipitated SERCA2a ($n=5$ in each group). Total phospholamban protein levels and phospholamban-serine 16 phosphorylation were assessed in response to intravenous application of 1000 pg isoproterenol. Unchanged CSQ protein levels were used for standardization (data not shown). * $P<0.05$ vs sham-NLC/WT; † $P<0.05$ vs corresponding NLC/WT-MI.

Moreover, protection from enhanced SR Ca^{2+} leak in STG-MI myocardium might be explained by a recent finding showing that S100A1 protein can decrease Ca^{2+} spark activity in ventricular cardiomyocytes under diastolic conditions.¹²

In contrast, failing SKO hearts exhibited severely disturbed SR Ca^{2+} fluxes. On a molecular level, we also observed an earlier downregulation of SERCA2 expression accompanied by increased nitration of the SR Ca^{2+} pump in failing SKO-MI myocardium. Several mechanisms might contribute at least in part to these deleterious defects. First, hemodynamic overload and pronounced hyperadrenergic activation most likely contribute to the accelerated decline of SERCA2 protein in failing SKO-MI hearts. Second, increased susceptibility of SERCA2 to nitrosative stress could also depend, at least in part, on the lack of cardiac S100A1 expression because S100A1, capable of interacting with the SR Ca^{2+} pump in a Ca^{2+} -dependent manner, has recently been shown to efficiently act as a Ca^{2+} -dependent NO acceptor and scavenger, respectively.²⁷ Finally, because S100A1 has been reported to stimulate SERCA2-mediated SR Ca^{2+} uptake,^{5,6,9–11} the simple absence of the activator might contribute to decreased SR Ca^{2+} uptake. Similar but less pronounced alterations were observed in infarcted control myocardium showing a persistent but progressive downregulation of S100A1 protein. In contrast, enhanced cardiac S100A1 protein levels might prevent these mechanistic alterations, and this is important to note because even small alterations of SERCA2 can alter SR Ca^{2+} loading, which in turn impairs myocardial function.²⁸

Subsequently, preserved contractile function in STG-MI hearts was mirrored by unaltered phospholamban-serine 16 phosphorylation, indicating that preserved *in vivo* β -adrenergic receptor-mediated inotropy partially relies on adequate diastolic SR Ca^{2+} refilling and systolic Ca^{2+} mobilization. SKO-MI hearts, however, exhibited an accelerated decrease in phospholamban phosphorylation and enhanced expression of GRK2 indicative of pronounced desensitized β -adrenergic receptor downstream signaling compared with infarcted control hearts.²⁹ Thus, our results strongly support the notion that preserved supranormal SR Ca^{2+} fluxes in STG-MI myocardium likely reflect an intact intracellular cardiac Ca^{2+} homeostasis of remote myocardium causative for preserved global contractile function.

Finally, we identified for the first time clinically relevant prohypertrophic neurohumoral signaling components conveying downregulation of cardiac S100A1 expression levels *in vitro*. Both Gq-protein-coupled receptor agonists as well as PKC activation seem to be sufficient to cause diminished S100A1 abundance, as seen in failing human myocardium. This is important to note because inhibition of Gq-dependent signaling clinically (eg, through ACE inhibitors) not only may reverse cardiac hypertrophy but also may prevent downregulation of cardiac S100A1 expression. Of note, we have previously shown that selective inhibition of Gq activation in the cardiac myocyte not only prevents pressure-overload ventricular hypertrophy³⁰ but also prevents maladaptive hy-



petrophy and cardiac dysfunction chronically.³¹ On the basis of our present findings, a contributing mechanism for the positive effects on the stressed heart after Gq inhibition in addition to blunted MAPK and deleterious signaling³² may be the preservation of S100A1 expression, which would preserve Ca²⁺ handling and contractile function in the postischemic heart. Eventually, intact cAMP-dependent downstream signaling might contribute to this effect because Gs-protein-coupled receptor and adenylyl cyclase agonists resulted in augmented cardiac S100A1 mRNA and protein levels. Further studies are warranted to delineate the molecular mechanism and physiological significance of in vitro reciprocal Gs- and Gq-dependent regulation of cardiac S100A1 expression.

In summary, by taking advantage of 2 different genetically manipulated mouse models with reciprocal cardiac S100A1 protein levels, our study provides unequivocal evidence for S100A1 as an indispensable regulator of the diseased heart to functionally adapt to pathophysiological stress in the course of post-MI HF. Given the fact that the search for crucial molecular mechanisms involved in the pathogenesis of HF is a current challenge of cardiovascular biology, our results further support the notion that alternative positive inotropic strategies such as S100A1, as it transitions from bench to bedside, might add a

promising novel target to the therapeutic armamentarium to prevent development and progression of HF or to restore contractile performance of failing myocardium.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Diminished cardiac S100A1 protein levels are characteristic of failing human myocardium, apparently irrespective of the underlying cause. However, the pathophysiological consequence of altered S100A1 protein levels for the development and progression of heart failure (HF) has not yet been explored. The present study addressed this clinically relevant issue and identified disturbed cardiac S100A1 expression as a key factor in the development and progression of HF and cardiac-related death. Thus, from a clinical perspective, preservation or restoration of normal cardiac S100A1 expression emerges as a powerful therapeutic strategy to prevent or treat human HF. Clinical relevance and feasibility of this strategy have recently been proven in experimental animal models of overt HF and acute myocardial infarction by the means of intracoronary viral S100A1 gene delivery, which rescued and prevented contractile failure and reversed cardiac remodeling. These results underscore the future clinical impact of S100A1 gene therapy with optimized viral shuttles, eg, adeno-associated viruses. Moreover, from a clinical standpoint, our study supports the notion that even established clinical drugs such as ACE inhibitors eventually exert their favorable effects in modifying the course of HF and regression of cardiac hypertrophy through abrogation of Gq-dependent downregulation of cardiac S100A1 expression. However, preservation of S100A1 expression in diseased human myocardium potentially underlying beneficial clinical effects of β -blockers, ACE inhibitors, or aldosterone antagonists awaits systematic evaluation. In summary, our translational approach highlights the pathophysiological relevance and underscores the tremendous therapeutic potential of S100A1 in the pathogenesis and future treatment of human HF.

Cardiac S100A1 Protein Levels Determine Contractile Performance and Propensity Toward Heart Failure After Myocardial Infarction

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