Plasticity of Surface Structures and $\beta_2$-Adrenergic Receptor Localization in Failing Ventricular Cardiomyocytes During Recovery From Heart Failure

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Background—Cardiomyocyte surface morphology and T-tubular structure are significantly disrupted in chronic heart failure, with important functional sequelae, including redistribution of sarcolemmal $\beta_2$-adrenergic receptors ($\beta_2$AR) and localized secondary messenger signaling. Plasticity of these changes in the reverse remodeled failing ventricle is unknown. We used AAV9.SERCA2a gene therapy to rescue failing rat hearts and measured z-groove index, T-tubule density, and compartmentalized $\beta_2$AR-mediated cAMP signals, using a combined nanoscale scanning ion conductance microscopy-Förster resonance energy transfer technique.

Methods and Results—Cardiomyocyte surface morphology, quantified by z-groove index and T-tubule density, was normalized in reverse-remodeled hearts after SERCA2a gene therapy. Recovery of sarcolemmal microstructure correlated with functional $\beta_2$AR redistribution back into the z-groove and T-tubular network, whereas minimal cAMP responses were initiated after local $\beta_2$AR stimulation of crest membrane, as observed in failing cardiomyocytes. Improvement of $\beta_2$AR localization was associated with recovery of $\beta$AR-stimulated contractile responses in rescued cardiomyocytes. Retubulation was associated with reduced spatial heterogeneity of electrically stimulated calcium transients and recovery of myocardial BIN-1 and TCAP protein expression but not junctophilin-2.

Conclusions—In summary, abnormalities of sarcolemmal structure in heart failure show plasticity with reappearance of z-grooves and T-tubules in reverse-remodeled hearts. Recovery of surface topology is necessary for normalization of $\beta_2$AR location and signaling responses. (Circ Heart Fail. 2012;5:357-365.)

Key Words: $\beta_2$-adrenergic receptors ■ transverse tubules ■ excitation-contraction coupling ■ heart failure ■ SERCA2a gene therapy ■ remodeling heart failure

Chronic heart failure is characterized by impaired $\beta$-adrenergic receptor ($\beta$AR) signaling in ventricular cardiomyocytes, resulting in reduced inotropic and lusitropic function. In addition to the functional changes, we and others have previously reported dramatic structural changes to the surface membrane and transverse tubule (TT) system in cardiomyocytes from chronically failing human and animal hearts. We recently reported that disruption of cell surface topography in failing cardiomyocytes alters the distribution of $\beta$ARs, with important functional sequelae. Specifically, the $\beta_2$-adrenergic receptor ($\beta_2$AR), usually located in the TT of healthy cardiomyocytes with spatially restricted cAMP cytoplasmic signaling microdomains, redistributes from the TT to the intergroove or membrane crest in chronically failing cardiomyocytes. In this case, stimulation of $\beta_2$ARs results in a greater spatial spread of the cAMP signal, matching that observed with $\beta_3$AR agonists. This move to global rather than local cAMP signaling of the $\beta_2$AR is suggested to be linked to loss of its relatively protective effect compared with the proarrhythmic proapoptotic $\beta_3$ARs.

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The TT system of the ventricular cardiomyocyte shows plasticity during development and disease, with a relatively underdeveloped structure in neonatal cardiomyocytes, reminiscent of the adult failing cardiomyocyte. During postnatal development, sarcomeric organization is laid down in the ventricular cardiomyocyte...
with the regular z-grooves and TT present in the healthy adult cell. TTs are reduced during the development of left ventricular hypertrophy, with further loss on progression to advanced chronic heart failure.4 TT disruption has been associated with increased spatial heterogeneity of calcium (Ca2+) transient initiation, leading to delayed time-to-peak for both the cytoplasmic Ca2+ transient and cardiomyocyte contraction.3

Recent advances in the treatment of chronic heart failure have led to dramatic improvements in the geometry and function of the failing left ventricle, a process called reverse remodeling. This has been observed with the increasing use of cardiac resynchronization therapy and/or left ventricular assist devices for advanced heart failure.10,11 These improvements at the level of the intact heart are matched by improvements in subcellular calcium cycling and excitation-contraction coupling. However, the changes to surface morphology and TT system during recovery from chronic heart failure, and their impact on surface receptor localization, remain unknown.

SERCA2a gene therapy represents a new therapeutic strategy for chronic heart failure, with left ventricular reverse remodeling reported in various animal models of heart failure12–14 and clinical benefits reported in the first phase 2 clinical trial.15 We recently reported recovery of chronic postinfarction rat heart failure model using a novel AAV9.SERCA2a vector, with reverse remodeling of abnormal calcium cycling, contractile function, and arrhythmia susceptibility.13 In the present study, we report both the changes in cardiomyocyte cell surface morphology and functional sequelae on β2AR signaling microdomains in cardiomyocytes from AAV9.SERCA2a-treated hearts, using the combination of high-resolution nanoscale (∼50 nm) hopping probe scanning ion conductance microscopy (HPICM) and Förster resonance energy transfer (FRET). We report that the recovery from chronic heart failure is accompanied by significant improvement of cell surface structure, with reappearance of the regular z-groove indentations and TT network, and by full or partial redistribution of β2AR into z-groove-TT signaling domains.

Methods
See the online-only Data Supplement for detailed methods.

Reverse-Remodeled Heart Failure Model
A chronic postinfarction rat heart failure model was used (HF) (n=22). A subgroup of HF rats (n=13) received a single intravenous injection of AAV9.SERCA2a at least 16 weeks after infarction as previously reported (HF+S),13 when the chronic heart failure phenotype is fully established. Age-matched, noninfarcted animals served as healthy nonfailing controls (n=8).

Studies were performed on ventricular cardiomyocytes isolated by enzymatic digestion from rat hearts 4 to 6 weeks after AAV9.SERCA2a gene therapy. Cardiac function assessed by pressure-volume analysis, and SERCA2a protein levels measured by immunoblotting, were recorded in a subgroup of hearts.13

Surface Morphology and Transverse Tubules
Freshly isolated cardiomyocytes were studied, using HPICM without continuous feedback.16,17 The z-groove index and T-tubule density were calculated.18 Correlation between surface structures and TTs was assessed using scanning surface confocal microscopy (SSCM).19 During SSCM, a laser is passed up a high numeric aperture objective and focused just below of the tip of the pipette, with a pinhole positioned at the image plane with the resulting confocal volume just below the pipette.17

Combined HPICM-FRET Studies: β2AR-Induced cAMP Transients and β2AR Localization
Simultaneous HPICM-FRET imaging of cAMP in living ventricular cardiomyocytes isolated from control, HF, and HF+S rats was performed after in vitro infection with Epac2-camps adenovirus for 48 hours, as previously described.7 HPICM allows accurate positioning of the scanning pipette to surface microdomains, for example, TT openings, with focal agonist application (1.6 pL/s delivered to <500 nm diameter surface region) and FRET recording allowing localization of functional β2AR-induced CAMP transients.7

β2AR Contractile Responses
Concentration-response studies to isoproterenol (ISO) were measured in isolated ventricular myocytes from control, HF, and HF+S rat hearts, using the IonOptix system.7

Calcium Transient Synchronicity
Electric field–stimulated cardiomyocyte Ca2+ transients were recorded.2 Standard deviation of the time from the beginning of the earliest Ca2+ subtransient to 50% of the peak amplitude (TT50 mol/L) for all subtransients was the measure of Ca2+ transient dysynchrony.

Western Blotting
Levels of SERCA2a, junctophilin-2 (JPH2), BIN1 (amphiphysin 2), and TCAP were measured, using immunoblotting, and corrected to GAPDH levels.

Statistical Analyses
Quantitative variables were compared using 1-way ANOVA with Tukey post hoc analysis, and ISO-stimulated sigmoidal dose–response curves were compared using the F test. Protein levels were compared between study arms using the nonparametric Mann-Whitney test. Nonlinear correlation (exponential growth) of TT:crest FRET ratio versus z-groove ratio was performed using GraphPad Prism 4.0. A value of P<0.05 determined statistical significance.

Results
Heart Failure Rescue by AAV9.SERCA2a
The postinfarction rat heart failure model displays an advanced failing phenotype with reduced myocardial SERCA2a expression.2 We used SERCA2a gene therapy to rescue our chronic heart failure model as this strategy allows effective reverse remodeling in an energetically favorable manner,12,13 and our previous studies show normalization of cytoplasmic calcium transient kinetics and amplitude, supporting plasticity of the functional arm of the structure-function relationship.13 In vivo AAV9.SERCA2a delivery improved steady-state and dynamic parameters of left ventricular systolic and diastolic function at 4 to 6 weeks after in vivo vector delivery in HF+AAV9. SERCA2a (HF+S) rats. Examples of pressure-volume loops from control, HF, and HF+S animals are given in Figure 1A. We previously reported increases in Emax (2.34±0.34 versus 1.27±0.13, P<0.01) and ESPVR (1.07±0.18 versus 0.58±0.06, P<0.05) and a reduction in EDPVR (0.06±0.01 versus 0.10±0.01, P<0.05) in a subgroup of these animals after AAV9.SERCA2a treatment (HF+S, n=6; HF, n=8).13

Normalization of Surface Morphology, Z-Groove Index, and TT Density After Recovery From Heart Failure
HPICM revealed a recovery of the alternating z-groove and crest morphology on the surface of HF+S cardiomyocytes to...
Figure 1. Reverse remodeling of the failing heart is characterized by recovery of sarcolemmal surface architecture and normalization of β2-adrenergic receptors (β2AR) location. A, Steady-state pressure-volume loops from healthy (blue), failing (black), and failing AAV9SERCA2a gene therapy (red) animals. B, Low-resolution (left) and high-resolution (center) hopping probe scanning ion conductance microscopy (HPICM) images from healthy controls (upper panels), failing (HF, middle panels), and failing AAV9SERCA2a-treated hearts (HF+S, lower panels), demonstrating recovery of z-groove contours and T-tubule openings after rescue by SERCA2a gene therapy. Right panels show cAMP Förster resonance energy transfer (FRET) YFP/CFP ratio traces recorded from whole cardiomyocytes after local β2AR stimulation in the cell crest (red) and in the T-tubule (black), demonstrating reversibility of functional β2AR location in the reverse-remodeled cardiomyocytes. Quantification of z-groove index (C) and the ratio of β2AR-stimulated cAMP signals from T-tubule:crest membrane (D) (mean ± SEM). Numbers of cardiomyocytes scanned/hearts and FRET ratios measured: control (C), n = 12/8 (10); HF, n = 26/9 (8); HF+S, n = 77/13 (11). **P < 0.01, ***P < 0.001. TT indicates transverse tubule; ISO, isoproterenol.
a pattern observed in healthy noninfarcted controls (Figure 1B). When quantified, this resulted in a significant increase in the z-groove index in HF/S cardiomyocytes compared with cardiomyocytes from untreated HF hearts (Figure 1C). Z-groove index correlates closely with TT structure (Figure S1), and di-8-ANNEPPS staining confirmed restoration of TT system in HF/S cardiomyocytes compared with HF controls, with normalization of the TT density (Figure 2).

**Normalization of β2-AR Localization and Signaling**

HPICM-FRET studies were performed in a subgroup of cardiomyocytes isolated from the left ventricle of HF, HF+S, and healthy control rats to analyze β2-AR localization and signaling to cAMP. β2-AR-mediated cAMP responses in HF myocytes were detected after focal (<500 nm) agonist application to both TT openings and crest membrane, as previously reported.7 In contrast, the β2-AR-mediated cAMP signals in HF+S cardiomyocytes were clearly detectable on local stimulation of the TT β2-AR population but absent (Figure 1B) after stimulation of the crest membrane β2-AR population. The HPICM-FRET responses in HF+S cells predominantly matched the functional β2-AR distribution seen in healthy ventricular cardiomyocytes (Figure 1B and online-only Data Supplement Figure II). The ratios of β2-AR-mediated FRET responses between TT and crest stimulated areas were significantly higher in HF/S cardiomyocytes compared with failing controls (Figure 1D). Correlation between z-groove index and the TT:crest ratio of β2-AR-mediated FRET responses demonstrated a logarithmic relationship with a threshold z-groove index value of ≈0.8, below which β2-AR distribution was equal or lower on TT

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**Figure 2.** Retubulation of failing cardiomyocytes after rescue by AAV9.SERCA2a gene therapy. A through C, Paired high-resolution simultaneous surface hopping probe scanning ion conductance microscopy (HPICM) (left) and confocal (right) images using scanning surface confocal microscopy (SSCM) technique from the surface of cardiomyocytes isolated from healthy (control, A), failing (HF, B), and failing+AAV9.SERCA2a-treated hearts (HF+S, C), demonstrating recovery of T-tubule openings. D, Quantification of T-tubule density, corrected to healthy baseline (mean±SEM; n=14 myocytes per study arm). *P<0.05, **P<0.01.
A feature of the reverse-remodeled heart is the recovery of the βAR sensitivity and contractile responses, which are significantly blunted in the failing heart. This may contribute to restoration of the Treppe effect in response to exercise or stress. We assessed the functional sequelae of βAR distribution and surface morphology normalization in cardiomyocytes by using βAR-stimulation with ISO. Baseline-corrected ISO concentration-response curves were normalized in HF+S cardiomyocytes compared with HF cardiomyocytes with respect to EC₅₀ and maximum amplitude and showed a significant improvement of contractile responses after AAV9.SERCA2a gene therapy (P<0.05; Figure 3C).

Retubulation Is Associated With Recovery of BIN 1 and TCAP Expression But Not JPH2
Myocardial SERCA2a proteins levels were reduced in the failing heart and significantly increased by AAV9.SERCA2a gene transfer, as previously reported. Loss of z-groove and TT structure in the failing hearts was associated with loss of a number of TT-associated proteins proposed to serve structural and anchoring functions, including JPH2, BIN1, and TCAP (Figure 4). Interestingly, SERCA2a gene therapy and cardiomyocyte retubulation were not accompanied by a recovery of JPH2 expression (Figure 4), suggesting that while important in normal development, JPH2 is not required for retubulation during reverse remodeling. In contrast, expression of BIN1, a membrane-binding protein associated with initiating membrane curvature and tubulogenesis in skeletal myocytes, was significantly increased in the retubulated SERCA2a-treated hearts (Figure 4). TCAP, a protein that anchors titin to muscle LIM protein at the z-disc and serves a central role in mechanotransduction, was also recovered in HF+S hearts.

Discussion
In the present study, we conducted for the first time nanoscale combined surface topology imaging and receptor localization in reverse-remodeled failing ventricular cardiomyocytes from a chronic heart failure model. We found that the complex ultrastructural cell surface architecture, with regular
z-grooves and the intracellular TT system, typically lost in chronic heart failure, is restored in SERCA2a gene therapy–treated hearts, demonstrating plasticity of these subcellular structural changes. This recovery and cardiomyocyte retubulation was accompanied by restoration of the normal surface location of functionally coupled β2ARs. These changes were associated with recovery of normal βAR-mediated inotropic responses and upregulation of both the myocyte tubulogen- esis protein BIN1 and the z-disc mechanosignaling protein JPH2, in SERCA2a-treated reverse-remodeled failing hearts.

Adult ventricular cardiomyocytes exhibit complex sarcolemmal membrane architecture with regular parallel grooves demarking intracellular sarcemeric intervals (z-grooves), and multiple tubular invaginations along such grooves form an intricate 3-dimensional tubular membrane network extending deep into the inner cellular core. This complex anatomy is critical for normal efficiency of excitation-contraction coupling and precise spatial organization of cell surface receptors. Disruption of the z-grooves and regular TT network are hallmarks of the failing cardiomyocyte phenotype, common to many animal models of chronic heart failure, and also reported in end-stage failing human heart.

Effective treatment of the chronically failing heart can lead to reversal of many of the maladaptive changes observed in chronic heart failure, a process known as “reverse remodeling.” Observations from cardiac resynchronization therapy and left ventricular assist device studies demonstrate the plasticity of the failing ventricle and the capacity for recovery and reverse remodeling at both organ and cellular levels. We and others have previously reported the dramatic reverse remodeling of the abnormalities of excitation-contraction coupling, SR calcium release and the calcium transient in failing ventricular cardiomyocytes from animals treated with SERCA2a gene therapy in vivo, and human end-stage failing ventricular cardiomyocytes transduced with SERCA2a in vitro. SERCA2a gene therapy trials for chronic heart failure are ongoing, with early reported results suggesting beneficial reverse remodeling as shown in animal studies. Understanding the molecular and cellular mechanisms underlying beneficial reverse remodeling may uncover new therapeutic targets for intervention in this patient population.

Given the importance of cell-surface architecture and the TT network to normal function, we wished to evaluate these structures in the failing ventricular cardiomyocyte from the reverse-remodeled rescued heart. We used SERCA2a gene therapy to rescue our chronic heart failure model as this strategy allows effective reverse remodeling in an energetically favorable manner, and our previous studies show normalization of calcium transient kinetics and amplitude, supporting plasticity of the functional arm of the structure-function relationship.

We report that, when assessed using high resolution HPICM, the sarclemmal structure with z-groove contours in failing ventricular cardiomyocytes were restored to normal levels in failing cardiomyocytes after SERCA2a gene therapy, confirming plasticity of these membrane structures and the capacity for retubulation of failing cardiomyocytes, indicative of cellular reverse remodeling. This was confirmed quantitatively by normalization of the z-groove index and TT density in cardiomyocytes isolated from HF+S hearts (Figures 1 and 2). This is supported by the recent reports of partial TT density recovery, both after exercise training in a similar HF model and also in right ventricular cardiomyocytes after sildenafil treatment in a monocrotaline-induced pulmonary hypertension model, albeit incompletely in comparison with the recovery observed here with SERCA2a gene therapy.

A feature of the reverse-remodeled heart is the recovery of the βAR sensitivity and contractile responses, which are significantly blunted in the failing heart. This recovery may contribute to restoration of the Treppe effect in response to exercise or stress. Several mechanisms underpin the reduced responsiveness to catecholamines in chronic heart failure, including reduction in the βAR/βAR-Gi ratio and increased βAR-Gi coupling; recently we reported that altered βAR location on the cardiomyocyte surface may also contribute to the reduced catecholamine-induced contractile response of the failing myocardium. Specifically disruption of sarclemmal architecture in chronic heart failure is accompanied by redistribution of β2AR-cAMP signaling from the restricted positions in the z-grooves of healthy cells to

![Figure 4. Retubulation is associated with recovery of myocardial BIN1 and TCAP expression but not JPH2. Immunoblots of myocardial SERCA2a, JPH2, BIN1, and TCAP expression in control (C), failing (HF), and failing hearts rescued with SERCA2a gene therapy (HF+S), with GAPDH as loading control. Quantitative comparison of SERCA2a, JPH2, BIN 1, and TCAP protein levels, showing reduction of all 4 proteins in the failing heart (black) compared with normal controls (blue), and recovery of SERCA2a, BIN 1, and TCAP after AAV8SERCA2a gene therapy (red) (mean±SEM except BIN1, median±interquartile range). Number of hearts: C, n=6; HF, n=5; HF+S, n=5. ***P<0.005, **P<0.01, *P<0.05, S, n=5. ISO indicates isoproterenol.](http://circheartfailure.ahajournals.org/Downloaded from)
locations across the cell surface in the intergroove (crest) membrane in failing cardiomyocytes. This has a number of important functional sequelae, with β₂AR subcellular cAMP signaling domains resembling those associated with the toxic features of β₁AR-Gs overstimulation, as well potential for loss of coupling with TT-located, L-type Ca²⁺ channels and enhanced coupling through Gi. Therefore, we wished to study the functional impact of this ultrastructural reverse remodeling on catecholamine responsiveness. Retubulation and normalization of sarcolemmal anatomy was accompanied by restoration of β₂ARs to their normal location within the surface TT openings in HF+S myocytes and loss of functional β₂ARs on the crest membrane, as seen in failing cardiomyocytes (Figure 1). Retubulation not only appears critical for normalization of basal ECC, but, through restoration of β₂AR location and coupling, it also contributes (along with any β₁AR changes) to the improvements in ECC responses to catecholamine stimulation, another physiological parameter impaired in the failing ventricular myocardium. ISO-stimulated βAR contractile responses reverted to normal levels in retubulated HF+S myocytes (Figure 3C), consistent with restoration of catecholamine-induced βAR-mediated inotropic responses, which are blunted in chronically failing hearts.

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The differences in structure and function across the study arms allowed us to examine the relationship between surface structure, quantified as z-groove ratio, and β₂AR location, expressed as the ratio of β₂AR-FRET-cAMP signals elicited from stimulation of receptors on the TT as compared with the intergroove crest membrane (TT:crest FRET ratio). The logarithmic correlation (Figure 3B) supports the hypothesis that regeneration of the z-groove and TT architecture is required for β₂AR relocation to the correct surface microdomain, which then permits appropriate coupling to normal Gs-dependent secondary messenger pathways. Retubulation therefore could contribute to recovery of catecholamine-induced βAR-mediated inotropic responses, which are blunted in chronically failing hearts.

Retubulation was associated with recovery of BIN1 (also known as amphiphysin 2) and TCAP expression but not JPH2, suggesting that BIN1 and/or TCAP could serve a role in tubulogenesis and stabilization of the de novo TT system (Figure 4). Previous studies suggest BIN1 as a likely candidate for tubulogenesis, given that BIN1 contains a Bin/Amphiphysin/Rvs (BAR) domain that allows direct phospholipid binding, and BIN1 overexpression in both CHO and skeletal muscle–derived cell lines induces formation of membrane tubules. Recently, a role for BIN1 in anchoring microtubules to the TT system has been reported, necessary for effective delivery of the L-type calcium channel to the TT orifice, identifying BIN1 as a candidate for both tubulogenesis, and targeting sarcolemmal receptor to specific cell surface microdomains. TCAP has been proposed to serve as a biomechanical transducer between the myofilament and z-disc macromolecular complex, and TCAP depletion in a zebrafish model leads to impaired transverse tubulogenesis.
during development, supporting the role for TCAP in the tubulogenesis observed with reverse remodeling after SERCA2a gene therapy. Xie et al noted recovery of JPH2 with partial restoration of TT in right ventricular cardiomyocytes from a rat model of pulmonary artery hypertension after treatment with sildenafil. This may reflect the different model (pulmonary artery hypertension with right ventricular hypertrophy versus left ventricular post–myocardial infarction HF), the differences in both chronicity and severity of the models (more severe in this post–myocardial infarction HF model), and the nature and duration of both treatments. Nevertheless, we demonstrate that in reverse remodeling of the left ventricle, JPH2 is not essential for restoration of a fully functional TT system, suggesting other pathways and scaffolding mechanisms exist to recreate the TT-SR interface.

Spatial organization of the TT system and close apposition to the sarcoplasmic reticulum is critical for efficient spatiotemporal coordination of excitation-contraction coupling. This spatial relationship is severely disrupted in chronic heart failure, leading to increased spatial heterogeneity in the delay of ECC, and the existence of “orphaned” ryanodine receptor populations, contributing to the reduced efficiency of ECC in heart failure. Retubulation and restoration of surface anatomy was associated with abrogation of the delayed upstroke of the cytoplasmic calcium transient (Figure 5C), and spatial coordination of triggered SR calcium release was restored to normal levels when measured as the spatial heterogeneity of transients in HF+SV cardiomyocytes (Figure 5B). This further extends the functional importance of restoring the TT system and subcellular ECC anatomy for the effective treatment of heart failure.

In summary, reverse remodeling of the failing heart using SERCA2a gene therapy is accompanied by significant remodeling of the cell surface structural abnormalities observed in failing ventricular cardiomyocytes, with important sequelae related to functional β2AR location. Importantly, our strategy was the reversal of an established heart failure rather than prevention of its development and adds to the increasing evidence that the viable cardiomyocytes in the failing heart are capable of significant rescue and the attainment of a more normal phenotype. Further research is required to dissect the molecular mechanisms underlying loss of TT network and z-grooves during disease progression and recovery of this architecture in reverse remodeled failing heart, which may lead to the development of new therapeutic strategies for the treatment of chronic heart failure.

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Disclosures
Dr Hajjar is the scientific founder of Celladon Inc, which is developing AAV1.SERCA gene therapy for therapeutic purposes.

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**CLINICAL PERSPECTIVE**

Chronic heart failure is characterized by reduced myocardial contractile performance and blunted catecholamine-mediated inotropic responses. We have previously reported that abnormalities of the ventricular cardiomyocyte cell surface architecture, including loss of 2-groove indentations and transverse tubules, and altered location of β2-adrenoceptors contribute to these functional abnormalities of the heart failure phenotype. In the present study, we demonstrate plasticity of the surface architecture, with recovery of both ventricular cardiomyocyte membrane microarchitecture and transverse tubule anatomy, as well as β2-adrenoceptor location, after rescue of a rat post–myocardial infarction chronic heart failure model using SERCA2a gene therapy. SERCA2a gene therapy is a novel strategy for advanced heart failure currently under assessment in clinical trials. These observed changes contribute to both the functional recovery and restoration of contractile responses and improved β-adrenoceptor sensitivity and reflect molecular and structural changes that are required for beneficial reverse remodeling during recovery from chronic heart failure.
Supplemental Material

Methods

All experiments were carried out in accordance with the United Kingdom Home Office Animals (Scientific Procedures) Act 1986, which conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Surface morphology and transverse tubules

Freshly isolated cardiomyocytes were studied using hopping probe ion conductance microscopy (HPICM) without continuous feedback. At each imaging point the pipette approaches the sample from a starting position above the surface features. The reference current is measured while the pipette is remote to the surface. The pipette approaches until the current is reduced by a predefined amount, usually 0.25–1%. The z-dimension actuator position when the current achieves this reduction is recorded as the height of the sample at this imaging point. Typically, even at a 1% reduction of the current, the pipette is still at a distance of about one inner pipette radius from the surface. The z-dimension actuator then withdraws the pipette away from the surface and the sample is moved laterally to the next imaging point. By continuously updating the reference current while the pipette is away from the surface, the method automatically adjusts for any slow drifts in the pipette current.

Calcium transient dyssynchrony

Stimulated cardiomyocyte Ca\(^{2+}\) transients were monitored as previously described.\(^1\) Temporal heterogeneity of the transients was assessed using a custom-made macro in ImageJ to separate the transients occurring along the long axis of the cell into smaller 3\(\mu\)m-sized portions (sub-transients).
Times were measured from the beginning of the earliest Ca\textsuperscript{2+} sub-transient to 50% of the peak amplitude (TT50M) for all sub-transients along the cell length. The standard deviation of all TT50M values was the measure of Ca\textsuperscript{2+} transient dyssynchrony. Time from 10%-90% peak amplitude was assessed using a custom-made macro in ImageJ. Results were averaged for 5-6 transients per cell for both dyssynchrony and rise-time measurements.

**Western blot**

Twenty micrograms of each sample were loaded in 4-20% acrylamide gel midi format (Invitrogen) and transferred on nitrocellulose membrane. After 1 hour blocking in 5% milk TBS-T, primary antibodies for JP2 (1/1000), BIN1 (1/500), TCAP (1/500) and GAPDH (1/5000) (respectively Invitrogen, Sigma-Aldrich, ProSci Incorporated and Millipore) were incubated overnight at 4 degrees. Appropriate secondary antibodies linked to HRP (ThermoScientific) were incubated for 1 hour at room temperature and blots were revealed with ECL (Millipore). Images were quantified by ImageJ software (NIH).

**References**

Figure S1. Relationship between z-groove index and T-tubule density for control (blue circles), failing (black circles) and failing + SERCA2a treated (red circles) cardiomyocytes. Linear correlation: $R^2=0.69$ p<0.05.
Figure S2. Quantification of the change in cAMP-FRET ratio after local β2AR stimulation in T-tubule or crest surface membrane microdomains of ventricular cardiomyocytes from HF+AAV9.SERCA2a hearts (n=8 cardiomyocytes). ***p<0.001.