Left Ventricular Dysfunction in Murine Models of Heart Failure and in Failing Human Heart is Associated With a Selective Decrease in the Expression of Caveolin-3

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ABSTRACT

Background: Caveolins are scaffolding proteins that are integral components of caveolae, flask-shaped invaginations in the membranes of all mammalian cells. Caveolin-1 and -2 are expressed ubiquitously, whereas caveolin-3 is found only in muscle. The role of caveolin-3 in heart muscle disease is controversial.

Methods and Results: The present study was undertaken to assess the effects of left ventricular dysfunction on the expression of caveolin proteins using 2 well characterized models of murine heart failure and failing human heart. Transgenic mice with constitutive overexpression of A1-adenosine receptor (A1-TG) demonstrated cardiac dilatation and decreased left ventricular function at 10 weeks of age. This was accompanied by a marked decrease in caveolin-3 mRNA and protein levels compared with non-TG control mice. The change in caveolin-3 expression was selective, because levels of caveolin-1 and -2 did not change. Confocal imaging of myocytes isolated from A1-TG mice demonstrated a loss of the plate-like appearance of T tubules. Caveolin-3 levels were also reduced in hearts from mice overexpressing tumor necrosis factor α. There was a direct relationship between caveolin-3 expression and fractional shortening in all mice that were studied (r = 0.65; P < .001). Although we could not demonstrate a significant decrease in caveolin-3 levels in failing human heart, we did find a direct correlation (r = 0.7; P < .05) between levels of caveolin-3 protein and Ca2+-adenosine triphosphatase, a marker of the heart failure phenotype.

Conclusions: These results suggest a relationship between left ventricular dysfunction and caveolin-3 levels and suggest that caveolin-3 may provide a novel target for heart failure therapy. (J Cardiac Fail 2011;17:253–263)

Key Words: Caveolin, heart failure, adenosine, tumor necrosis factor.

Caveolae are 50–100-nm flask-shaped lipid rafts that appear as invaginations of plasma membranes in almost all types of cells. In the heart, studies using fluorescent calcium probes have demonstrated that calcium waves originate from caveola-rich areas on the surface of endothelial cells,1,2 and calcium channels and important

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Manuscript received June 28, 2010; revised manuscript received October 7, 2010; revised manuscript accepted October 25, 2010.

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Supported by National Heart, Lung, and Blood Institute grants HL 091799-01, HL 061690, HL 085503, HL 075443, HL 58672, and HL 74854.

See page 262 for disclosure information.

1071-9164/$ - see front matter © 2011 Elsevier Inc. All rights reserved. doi:10.1016/j.cardfail.2010.10.008
cardiac regulatory proteins, including G protein-coupled receptors, have been localized to caveolae. The unique shape of caveolae is attributable to the presence of the scaffolding protein caveolin. When caveolin is expressed in the membrane it spontaneously organizes membrane lipids into caveolae. Caveolins also anchor receptors in the caveolae and are crucial to receptor internalization in response to ligand stimulation, receptor recycling, and compartmentalization of downstream signals.

Three distinct mammalian caveolin genes have been identified: Caveolin-1, -2, and -3. Caveolin-1 and -2 are expressed predominantly in endothelial cells and adipocytes, whereas caveolin-3 is confined to skeletal, cardiac, and smooth muscle.

Although caveolins play a significant role in the physiology of the heart, studies designed to understand their role in cardiovascular pathophysiology have resulted in incongruent findings. Chronic hypoxia and chronic beta-adrenergic receptor stimulation result in down-regulation of caveolin expression. In addition, caveolin-3 expression is decreased in a rat model of hypertension-induced cardiomyopathy, and normalization of caveolin-3 levels was associated with improved exercise and regression of the hypertrophic phenotype. Consistent with the finding that caveolin-3 expression decreased in models of heart failure, genetic ablation of caveolin-3 in mice resulted in the development of a heart failure phenotype with hypertrophy, dilation, and reduced ventricular function.

To better understand the relationship between left ventricular dysfunction and caveolin expression, we took advantage of 2 well characterized murine models of left ventricular dysfunction and caveolin expression. In the mouse, chronic hypoxia and chronic beta-adrenergic receptor stimulation result in down-regulation of caveolin-3 expression. Similarly, failing human heart obtained from patients with a Pro104Leu mutation in caveolin-3. Dogs with pacing-induced heart failure demonstrated increased levels of caveolin-3 protein.

In contrast, Ohswara et al. described cardiac hypertrophy and enhanced contractility—not dilation and failure—in transgenic mice with a Pro104Leu mutation in caveolin-3. Dogs with pacing-induced heart failure demonstrated increased levels of caveolin-3 protein. Similarly, failing human heart obtained at the time of the placement of a left ventricular assist device demonstrated increased caveolin-3 mRNA compared with nondiseased human heart tissue, although levels of caveolin-3 increased further after left ventricular unloading.

To better understand the relationship between left ventricular dysfunction and caveolin expression, we took advantage of 2 well characterized murine models of left ventricular dysfunction: transgenic mice with constitutive (TGcon) or induced (TGind) cardiac-restricted overexpression of the human A1-adenosine receptor (AR; A1-TG) and mice with cardiac-restricted overexpression of TNF-α (FCS). Cell culture was adjusted to pH 7.0 by the addition of sodium bicarbonate. Two-dimensional transthoracic echocardiography was performed on WT and transgenic mice by use of the Visualsonics VeVo 770 imaging system with a 707 scanhead. Left ventricular dimensions were measured in M-mode short-axis view in end-diastole and end-systole. Mice were lightly anesthetized with inhalation of isofluorane (1.5%), placed in supine position, and restrained; total anesthesia duration did not exceed 5 minutes.

Methods

Transgenic Mouse Generation

Transgenic mice with constitutive (TGcon) and adult-induced (TGind) cardiac-restricted overexpression of the human A1- and A2A-AR were generated as previously described. Doxycycline (Dox) was deleted from the diet of pregnant breeders (TGcon) or from transgenic offspring at 3 weeks of age (TGind). Transgenic mice with cardiac-restricted overexpression of TNF-α in FVB background mice (TNF1.6) were generated as previously described. The A1-AR mouse line was crossed with transgenic GtCt mice with cardiac-specific inducible inhibitor of zGt2 function to generate the A1GtCt triple-transgenic mouse lines.

Echocardiography

Two-dimensional transthoracic echocardiography was performed on WT and transgenic mice by use of the Visualsonics VeVo 770 imaging system with a 707 scanhead. Left ventricular dimensions were measured in M-mode short-axis view in end-diastole and end-systole. Mice were lightly anesthetized with inhalation of isofluorane (1.5%), placed in supine position, and restrained; total anesthesia duration did not exceed 5 minutes.

Real-Time Quantitative Polymerase Chain Reaction

Complementary DNA was reverse transcribed from 1 μg of total RNA extracted from mouse left ventricles (n = 5 per group) to determine expression of endogenous caveolin-3. Two sets of caveolin-3 primers were validated and used: Fj, 5′-CCG ACA CAG ATC TGG-3′ (Exon 1)/Rj, 5′-GAG CAG GGC CTG CCC CCA-3′ (Exon 2); and Fs, 5′-CTG CCC CCA GGA CTA TCA AC-3′ (Exon 1)/Rs, 5′-TTC CAG ATC CGT GTG CTC TTC-3′ (Exon 2). GAPDH and actin genes were used as references for normalization of obtained measurements. Analysis of gene expression level was performed using the 2−ΔΔCt method.

Isolation and Adenoviral Infection of Adult Murine Cardiac Myocytes

Adult myocytes were isolated as previously described. Briefly, septum and left ventricular free wall from male 12–14-week TG hearts or wild-type (WT) non-TG control hearts were enzymatically digested with collagenases B and D and protease XIV. Isolated adult myocytes were plated on laminin-coated glass coverslips in a 6-well plate, and the Ca2+ concentration of the buffer was progressively increased from 0.05 to 0.125 to 0.25 to 0.5 mmol/L in 3 steps (10 min interval between each). The 0.5 mmol/L Ca2+ buffer was then aspirated and replaced with minimal essential medium (Sigma M1018) containing 1.2 mmol/L Ca2+, insulin-transferrin-selenium supplement (Invitrogen, Carlsbad, CA), 1% penicillin-streptomycin and 2.5% fetal calf serum (FCS). Cell culture was adjusted to pH 7.0 by the addition of NaHCO3 (0.57 g/L) and incubated in a 3.5% CO2 incubator for 1 hour. Then, cell culture medium was replaced with fresh medium without FCS and infected with either adenovirus (Adv) expressing green fluorescent protein (GFP) or Adv expressing both caveolin-3 and GFP at a multiplicity of infection of 50. After 3 hours’ incubation, viral media were removed and myocytes or H9C2 cells were cultured for 2 days.
Immunoblot Analysis

Left ventricular protein expression was detected by immunoblotting with an Odyssey scanner (Li-Cor, Lincoln, Nebraska). Left ventricular tissue was lysed with a modified RIPA buffer (20 mmol/L Tris-HCl [pH 7.6], 137 mmol/L NaCl, 10% glycerol, 1% NP40, 0.1% sodium dodecyl sulfate (SDS), 0.5% Na-deoxycholate) supplemented with protease and phosphatase inhibitors (in mmol/L: 10 NaF, 10 PMSF, 1 NaVO4, 1 NapyroPO4, 1 EDTA, 1 DTT, 5 μg/mL leupeptin; and 5 μg/mL aprotinin). Twenty μg protein lysates was separated by electrophoresis in a 4%–12% Bis Tris gel, transferred onto nitrocellulose, and probed with primary antibodies. Blots were subsequently incubated with secondary antibodies. Bands were visualized and directly quantified with the Odyssey v1.2 software. The following antibodies were used: anti-A1-AR (Affinity BioReagents, Rockford, Illinois), anticaveolin-1, -2, and -3 (BD Transduction Laboratories, San Jose, California), anti-pAkt, -pErk, -pJNK and -p-P38 (Cell Signaling Technology, Beverly, Massachusetts), ant amyogenin and anti-ID2 (Santa Cruz Biotechnology, Santa Cruz, California), and anti-GAPDH (Fitzgerald Industries International, Concord, Massachusetts); secondary antibodies: goat antimouse Alexa Fluor 680 (Invitrogen) and IRDye 800 goat antirabbit (Rockland, Gilbertsville, Pennsylvania).

Immunofluorescence Microscopy

Immunofluorescence myocyte staining, microscopy procedures, and image acquisition were performed as previously described. The primary antibody used was anticaveolin-3 (BD Transduction Laboratories). The secondary antibody used was Alexa-488 conjugated antimouse (Invitrogen) with 350 nmol/L DAPI nuclear stain (Invitrogen).

Myocyte T-Tubule Staining

Isolated myocytes were stained with di-8-ANEPPS (10 μmol/L) at 37°C for 10 minutes. Stained myocytes were washed and examined by confocal microscopy (Zeiss LSM 510), using an excitation/emission wavelength ratio of 488/505 nm.

Image Processing

Digital images of individual myocytes were processed by using a fast Fourier transform (FFT) algorithm to quantify the organization of caveolin-3 and T-tubule signal within cardiac myocytes. Each cell image was rotated horizontally to align periodic T-tubules in a vertical direction. The diffraction pattern with order spectra was displayed horizontally when FFT was performed on the image. Owing to discontinuities and cell wall in the image, a small region of interest (200 × 60 pixels) with the best caveolin-3 and tubule image was selected from each image sample for WT and A1-TGcon groups. The image contrast was enhanced by unsharp mask to increase acutance, and then the FFT algorithm was applied in IDL digital imaging software (Germantown, Wisconsin). Power spectrum in log scale versus spatial frequency was calculated for each image.

Electron Microscopy

Hearts were collected from male 12–14-week WT (n = 3) and A1-ARcon TG (n = 3) mice and fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4. After 15 minutes of initial fixation, a 1-mm longitudinal slice and a 1-mm transverse slice were made through the left ventricle with the aid of Acrylic Heart Matrices (Harvard Apparatus, Holliston, Massachusetts). The tissue slices were further dissected and fixed for a total of 2 hours at room temperature. Postfixation was performed with 1% osmium tetroxide in 0.1 mol/L sodium odylate buffer, pH 7.4, for 1 hour, followed by dehydration through graded alcohols and propylene oxide. The samples were embedded in Embed 812 (Electron Microscopy Sciences, Hatfield, Pennsylvania). Longitudinal and transverse sections were cut on an Ultratcut E ultramicrotome (Reichert-Jung [now Leica], Wein, Austria) and stained with uranyl acetate and lead. Images were collected with an AMT XR41-B 4 megapixel camera on a Hitachi H-7000 electron microscope.

Human Heart Tissue Analysis

Nonfailing (n = 5) human hearts were obtained from unused organ donors with no history of cardiac dysfunction or coronary artery disease. Failing hearts (n = 19) were obtained from end-stage cardiac transplant recipients with advanced ischemic or idiopathic dilated cardiomyopathies. The Institutional Review Boards of Thomas Jefferson University, University of Pittsburgh, and University of Colorado Health Sciences Center approved the study. Approximately 100 mg left ventricular free wall tissues frozen at −80°C were extracted with 300 μL modified RIPA buffer (20 mmol/L Tris-HCl [pH 7.6], 137 mmol/L NaCl, 10% glycerol, 1% NP40, 0.3% SDS, 0.5% Na-deoxycholate supplemented with Thermo Fisher Scientific Halt Protease and Phosphatase Inhibitor Single-Use Cocktail). The homogenates were centrifuged at 13,000g for 20 minutes at 4°C. Protein were heated and denatured with sample buffer, then stored at −80°C for immunoblotting analysis. Total cardiac tissue homogenates (40 μg in a volume of 20 μL) were immunoblotted. The following antibodies were used: anticaveolin-3 (BD Transduction Laboratories), αH-subunit of T-type calcium channel (Neurorhab, UC Davis 75-095), antiactin (Sigma-Aldrich, St Louis, Missouri), and anti-GAPDH and caveolin-1 (Santa Cruz Biotechnology).

Statistical Analysis

A commercial software package was used for statistical analysis (Graph Pad Software, La Jolla, California). Comparison of means ± SEs was analyzed by nonparametric Mann-Whitney test. Non-parametric Spearman rank test was used to correlate caveolin-3 expression and fractional shortening in mice. Multiple regression analysis was used to correlate human SERCA expression with caveolin-1, caveolin-3, αH-subunit of T-type calcium channel, and actin. A P value of < .05 was considered to be statistically significant.

Results

As we have demonstrated previously, mice constitutively overexpressing A1-AR (A1-TGcon) developed marked cardiac dilatation and a profound decrease in left ventricular contractility by 10 weeks of age (Fig. 1A; Table 1). To test the hypothesis that abnormalities in cardiac function might be associated with changes in caveolin-3 protein, we assessed caveolin-3 protein and mRNA levels in 10-week-old WT and A1-TGcon left ventricular myocardium. A1-TGcon myocardium had significantly less caveolin-3 protein than WT control samples (WT 1.00 ± 0.05 versus A1-TGcon 0.39 ± 0.07; P < .05; Fig. 1B). However, no
significant changes were seen in the levels of caveolin-1 or -2 protein (data not shown). Similarly, levels of caveolin-3 mRNA were also reduced in A1-TGcon myocardium (Fig. 1C). Immunofluorescence staining of caveolin-3 in isolated myocytes showed that whereas caveolin-3 in WT myocytes stained along the striated T-tubule system, caveolin-3 in A1-TGcon myocytes could not be visualized along the T-tubules (Fig. 1D).

Transmission electron microscopy was performed to evaluate the morphology of A1-TGcon mice. Heart samples viewed in both longitudinal and transverse planes revealed distinctive dilation of the T-tubules in the A1-TGcon mice (Fig. 2A). Focal accumulations of membranous material in whirled and concentric formations (myelin figures) were also visualized in the A1-AR TG hearts but not in the WT control samples (data not shown). To further evaluate T-tubule structure, T-tubules in live myocytes were marked with di-8-ANNEPS, a membrane phospholipid-binding dye. As seen in Figure 2B, unlike T-tubules in WT myocytes, T-tubules in A1-TGcon myocytes were not organized in a normal cross-striation pattern.

We quantified abnormal T-tubule organization by FFT analysis. The distance between the zero- and first-order diffraction spots was inversely proportional to the length of repeating units in the grating, ie, the sarcomere length. FFT of di-8-ANNEPS-stained confocal images gave rise to a series of well defined diffraction spots in WT myocytes (Fig. 2B, left lower panel). In contrast, first-order diffraction spots were missing in cells from A1-TGcon myocytes, suggesting that the T-tubules were disorganized.

The expression of the A1-AR transgene can be inhibited by maintaining the mice on Dox. Removal of Dox-containing diets from mice at 3 weeks of age induced A1-AR gene expression at 6 weeks of age. At 10 weeks of age, mice with induced overexpression of A1-AR (A1-TGind) had a fractional shortening (Fig. 3A) similar to that of litter-mate control animals. However, at 26 weeks of age, A1-TGind mice demonstrated a significant decrease in left ventricular function (percentage of fractional shortening [%FS]: WT 47 ± 2 vs A1-TGind 30 ± 2; P < .05; Fig. 3A; Table 1) which was similar to that seen in mice with constitutive overexpression of A1-AR. As seen in Fig. 3B, 26-week-old A1-TGind mice demonstrated a marked decrease in caveolin-3 expression, whereas aging of WT mice from 12 weeks to 26 weeks did not alter caveolin-3 expression. Caveolin-3 levels were also reduced in mice overexpressing the proinflammatory protein TNF-α (Fig. 3C). These mice show a more modest decrease in left ventricular function than is found in mice with constitutive overexpression of A1-AR. There was a statistically significant correlation (r = 0.65; P < .001) between FS and caveolin-3 expression in A1-TGcon, A1-TGind, TNF1.6, and appropriate age-matched and WT control animals (Fig. 3D).

Because caveolins are known to regulate signal transduction, we determined activation status of signaling proteins Akt, Erk, Jnk, and p38 in WT and A1-TGcon left ventricular myocardium. Immunoblotting of these phosphokinases showed that only phosphorylated Akt was reduced in an A1-TG-dependent manner (Fig. 4A and B). To determine
if Caveolin-3 expression was sufficient to rescue Akt activity in failing myocytes overexpressing A1-AR, myocytes were isolated from 20-week-old WT and adult-induced A1-TG mice and infected with either Adv

caveolin-3 or Adv-GFP. After culturing infected myocytes for 48 hours, cells were stimulated with insulin for 15 minutes. As seen in Fig. 4C, caveolin-3 overexpression synergized with insulin to phosphorylate Akt and enhanced the phosphorylation of the Akt kinase target P-Ras40 in myocytes isolated from A1-TG mice (n = 4; P < .05).

To determine whether alterations of caveolin-3 expression and organization were specific to A1-AR overexpression, we assessed caveolin-3 expression in mice overexpressing another adenosine receptor subtype, A2A, at levels similar to those seen in the A1-TGcon model.13,14

In marked contrast to the constitutive overexpression of A1-AR, overexpression of A2A-AR did not result in changes in caveolin-3 protein levels (Fig. 5A) or caveolin-3 expression pattern (Fig. 5B). As we reported previously, co-overexpression of A1-AR and A2A-AR at an equimolar ratio prevented the development of left ventricular dysfunction (Fig. 5C), and these bitransgenic animals did not demonstrate alterations in the levels of caveolin-3 protein (Fig. 5A).

To understand whether the changes in caveolin-3 protein in murine models of heart failure were representative of

| Table 1. Echocardiography Data of WT, A1-TGcon, and A1-TGind Mice |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| n                          | 8 | 10 | 6 | 5 |
| HR (bpm)                   | 441 ± 16 | 316 ± 24** | 151 ± 7** | 162 ± 13** |
| %FS                        | 46.5 ± 1.7 | 49.2 ± 2.6 | 29.7 ± 1.6* | 16.8 ± 4.7** |
| LVEDD (mm)                 | 2.84 ± 0.11 | 3.18 ± 0.11 | 4.91 ± 0.24** | 4.91 ± 0.21** |
| LVESD (mm)                 | 1.53 ± 0.08 | 1.64 ± 0.13 | 3.45 ± 0.24** | 4.10 ± 0.36** |

Echocardiography of male wild type (WT) and mice constitutively expressing A1-adenosine receptor (AR) (A1-TGcon) and after doxycycline was removed from their diet at 3 weeks to induce myocardial A1-AR expression (A1-TGind). HR, heart rate; %FS, percentage of fractional shortening; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension. Values are presented as mean ± SEM.

*P < .01 versus WT.

**P < .001 versus WT.

Fig. 2. (A) Electron microscopy images of myocardial sections of male 12–14-week WT (n = 3) and A1-TGcon (n = 3) mouse hearts. T-Tubules (arrows) are dilated in the transgenic cardiac muscle. The dilated T-tubules contain membranous and basement membrane–like material; m, mitochondrion. Bar = 1 μm. (B) T-Tubules were stained with the voltage-sensitive lipid dye di-8-ANNEPS in live WT and A1-

Caveolin-3 and LV Dysfunction / Feiner et al 257
Fig. 3. (A) Adult A$_1$-AR induction. Doxycycline was removed from transgenic mice at 3 weeks of age to induce A$_1$-AR expression (A$_1$-TG$_{ind}$). Percentage of fractional shortening (%FS) was determined in A$_1$-TG$_{ind}$ mice at 10 and 26 weeks of age. *$P < .05$ versus 10-week-old A$_1$-TG$_{ind}$. (B) Caveolin-3 (Cav3), A$_1$-AR, and GAPDH expression in 10–12-week-old WT (n = 8), 20–26-week-old WT (n = 4), 10–12-week-old A$_1$-TG$_{ind}$ (n = 4), and 20–26-week-old A$_1$-TG$_{ind}$ (n = 8) mouse left ventricles. Data shown are mean ± SE. *$P < .05$. (C) Caveolin-3 and GAPDH expression in 12-week-old WT and TNF1.6 left ventricular tissues (n = 4). Data shown
changes that might occur in the failing human heart, we measured caveolin-3 expression in samples of failing human heart obtained at the time of cardiac transplantation and compared these with samples of nonfailing human heart tissue obtained at the time of tissue harvest. The mean age of the individuals from whom the nonfailing hearts were harvested was 50.4 ± 3.0 years (n = 5) and that of the transplant recipients 54.7 ± 1.5 years (n = 20; P = NS). There was a trend toward a decrease in caveolin-3 levels in failing human heart normalized to GAPDH (1.2 ± 0.2 units) compared with nonfailing control samples (1.9 ± 0.6 units), but this trend did not reach statistical significance, owing to high variation within the failing-heart group (P = .24). The Ca\(^{2+}\)-adenosine triphosphatase (SERCA)/GAPDH ratio in the failing cohort (0.80 ± 0.07; n = 18) was significantly lower than in nonfailing control hearts (1.14 ± 0.07; n = 5; P < .05). We also found that there was a direct correlation between myocardial levels of caveolin and SERCA, an established molecular marker of the heart failure phenotype (Fig. 6). Using multiple regression analysis, levels of caveolin-1, α1H-subunit of T-type calcium channel and actin did not correlate with levels of SERCA.

A1-AR mediates at least some of its downstream effects by coupling with the inhibitory G protein, Gi. To test whether the A1-AR–mediated decrease in caveolin-3 expression was mediated by Gi, we created TG mice overexpressing both A1-AR and a Gi-selective inhibitory peptide...
GiCT is composed of the carboxyl-terminal 63 amino acids of Gi2 to “functionally knock out” all G protein–coupled receptor/Gi–induced signals in the heart.17 As seen in Figure 7, mice that expressed both A1-AR and GiCT demonstrated marked cardiac dilatation and diminished left ventricular function compared with A1-TGind mice (P < .001), suggesting that functional knockout of cardiac Gi signaling (GiCT) could not rescue A1-AR signaling-induced cardiomyopathy. The transcription factor myogenin is both sufficient and necessary to activate caveolin-3 expression by binding to its regulatory site in the promoter region of the caveolin-3 gene. In contrast, the nuclear protein Id2 inhibits myogenin activity. We were not able to demonstrate changes in the levels of myogenin or Id2 protein in A1-TGcon mice compared with WT control samples (data not shown).

**Discussion**

In this study, we demonstrated that left ventricular dysfunction in mice overexpressing A1-AR is associated with significantly reduced levels of caveolin-3 mRNA and
Confocal microscopy of isolated myocytes immunostained with caveolin-3 and di-8-ANNEPS showed abnormal caveolin-3 signal organization and T-tubules in myocytes isolated from A1-TG mice. Similarly electron microscopy demonstrated T-tubule dilation with focal accumulation of membranous material. These changes were not selective for A1-Ae-induced heart failure, because abnormalities in caveolin-3 expression were also observed in a commonly studied form of murine heart failure, cardiomyopathy secondary to constitutive overexpression of TNF-α,20 as well as in failing human heart. In TG mouse myocardium with augmented expression of the highly homologous A2A-AR, caveolin-3 levels were normal, suggesting that decreased expression of caveolin-3 is unlikely to be due to overexpression of a G protein–coupled receptor per se. In addition, co-overexpression of A1- and A2A-AR did not result in abnormalities in left ventricular function or in caveolin-3 expression or localization, and these animals had near-normal ventricular function.14 Interestingly, inhibition of Gi2 function (GiCT) did not rescue A1-AR-induced cardiomyopathy, suggesting that the change in caveolin-3 expression was not mediated by Gi.

That changes in levels of caveolin-3 protein can alter cardiac function has been demonstrated in earlier studies. Both calcium cycling and A1-AR have been previously localized to caveolae and functionally linked to caveolin-3. Calcium influx and regulation have been localized to caveolae in the myocardium.21 Studies using fluorescent calcium probes demonstrated that calcium waves originate from caveolae rich areas on the cell surface,1,2 and calcium channels and regulatory proteins have been localized to caveolae.3 Furthermore, caveolins, the proteins that anchor receptors in the caveolae, are crucial in A1-AR internalization in response to ligand stimulation, receptor recycling, and downstream signal compartmentalization.4,5 In fact, A1-AR communoprecipitated with caveolin-3 and likely localizes to caveolae in the cell membrane.4 Mice overexpressing TNF-α also demonstrate profound changes in calcium homeostasis.22 Thus, the profound reduction in caveolin-3 levels in hearts of A1-TG and TNF1.6 mice could contribute to ventricular dysfunction in both murine heart failure models.

We were unable to demonstrate a significant decrease in the levels of caveolin-3 in failing human heart compared with nonfailing control samples. This was likely due to the heterogeneity that is found in both failing and
“nonfailing” human hearts. However, we were able to demonstrate a highly significant and linear relationship between the levels of caveolin-3 and SERCA, an incontrovertible marker of the adult heart failure phenotype. This relationship was specific for caveolin-3, because a similar relationship could not be demonstrated with caveolin-1, actin, or z1H-subunit of T-type calcium channel.

Abnormalities in caveolin-3 expression have been previously associated with myocardial dysfunction, but there are significant inconsistencies in the results. Caveolin-3−/− knockout mice developed a cardiomyopathic phenotype with significant hypertrophy, dilation, and reduced fractional shortening of the myocardium.9 Similarly, caveolin-3 expression was decreased in hypertension-induced cardiomyopathy, and normalization of caveolin-3 levels was associated with improved exercise tolerance and regression of the hypertrophic phenotype in a rat model.8 In contrast, Ob-sawa et al10 described hypertrophic cardiomyopathy and enhanced—not diminished—contractility in transgenic mice with a Pro104Leu mutation in caveolin-3. Similarly, Hayashi et al13 discovered a mutation in a residue involved in limb girdle muscular dystrophy in a sibling case of hypertrophic cardiomyopathy. Both caveolin-1 and -3 protein levels increased in dogs with heart failure secondary to overdrive pacing as measured in arbitrary units on 2 Western blots, and electron microscopy and 2-photon imaging showed a normal striated pattern for caveolin-3 consistent with localization in T-tubules.11 Caveolin-3 mRNA levels were reported to be elevated in human heart tissue obtained at the time of placement of a left ventricular assist device compared with nonfailing control samples.12 The disparities between the various data may be due to the use of different species, the failure to assess levels of protein (versus levels of mRNA), or differences in the degree of left ventricular dysfunction seen in the different models. Unlike earlier studies, we used multiple heart failure models and varying degrees of left ventricular dysfunction, which allowed us to demonstrate that changes in caveolin-3 expression are only identifiable in the late stage of left ventricular dysfunction.

That a decrease in caveolin-3 expression could fundamentally change the morphology of the heart was demonstrated by the finding that overexpression of A1-AR and abnormal caveolin-3 were associated with disorganization of T-tubule structure, as observed by confocal microscopy, and T-tubule dilation and focal accumulation of membranous material in dilated T-tubules, as seen by electron microscopy. This finding is consistent with earlier studies showing that caveolin-3 is a crucial structural component of the caveola membrane and regulates membrane-repair proteins, such as dysferlin.24,25

Overexpression of A1-AR also resulted in a marked decrease in phosphorylated Akt. Phosphorylation of Akt protects the heart from injury during ischemia-reperfusion.26–28 Mice deficient in Akt subtypes demonstrate myocardial contractile dysfunction after constriction of the ascending aorta and enhanced apoptosis after ischemic injury.29,30 Our finding that overexpression of caveolin-3 in A1-TG myocytes could effectively reverse the decrease in Akt phosphorylation suggests that the decrease in Akt phosphorylation in mice overexpressing the A1-AR is secondary to a marked decrease in the level of caveolin-3. It remains to be determined whether this decrease in phosphorylation of Akt is the result of altered caveolin-3 signaling or due to an uncoupling of relevant signaling molecules from their target proteins due to changes in caveola structure secondary to the marked decrease in caveolin-3 protein. In several models of cardiomyopathy with disparate etiology, A1-AR overexpression and TNF-α overexpression demonstrated reduced caveolin-3 expression. It is possible that reduced caveolin-3 and T-tubular disorganization may be a general pathologic mechanism in the progression of the heart failure phenotype.

Further evaluation of the complex pathways that regulate caveolin-3 expression and caveolin-3−/−dependent signaling may provide important new therapeutic insights.

Two findings suggest that left ventricular dysfunction results in altered caveolin-3 expression through a unique and as yet undefined mechanism. First, functional inhibition of Gi, a primary pathway for A1-AR signaling as well as for regulating gene expression, failed to attenuate the effects of A1-AR overexpression on levels of caveolin-3. Second, the decrease in caveolin-3 expression in mice with left ventricular dysfunction was not associated with changes in the level of myogenin, a transcription factor that is both sufficient and necessary for activation of caveolin-3 expression, nor did it alter levels of Id2, a nuclear protein that regulates myogenin activity.31 Because we saw parallel changes in both caveolin-3 mRNA and its protein, it is unlikely that these changes can be attributable to posttranslational modification. One possible mechanism is that caveolin-3 gene transcription is differentially regulated in heart failure by microRNAs. Further studies will be required to test this hypothesis.

Conclusions

The results of the present study demonstrate that the heart failure phenotype in A1-AR over-expressing transgenic mice and in mice over-expressing TNF-α is associated with a marked decrease in myocardial levels of caveolin-3, and loss of T-tubule structural integrity. Taken together, these results suggest that caveolin-3 may be a novel therapeutic target in patients with heart failure.

Disclosures

None.

References


