α-E-Catenin Inactivation Disrupts the Cardiomyocyte Adherens Junction, Resulting in Cardiomyopathy and Susceptibility to Wall Rupture

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Background—α-E-catenin is a cell adhesion protein, located within the adherens junction, thought to be essential in directly linking the cadherin-based adhesion complex to the actin cytoskeleton. Although α-E-catenin is expressed in the adherens junction of the cardiomyocyte intercalated disc, and perturbations in its expression are observed in models of dilated cardiomyopathy, its role in the myocardium remains unknown.

Methods and Results—To determine the effects of α-E-catenin on cardiomyocyte ultrastructure and disease, we generated cardiac-specific α-E-catenin conditional knockout mice (α-E-cat cKO). α-E-cat cKO mice displayed progressive dilated cardiomyopathy and unique defects in the right ventricle. The effects on cardiac morphology/function in α-E-cat cKO mice were preceded by ultrastructural defects in the intercalated disc and complete loss of vinculin at the intercalated disc. α-E-cat cKO mice also revealed a striking susceptibility of the ventricular free wall to rupture after myocardial infarction.

Conclusions—These results demonstrate a clear functional role for α-E-catenin in the cadherin/catenin/vinculin complex in the myocardium in vivo. Ablation of α-E-catenin within this complex leads to defects in cardiomyocyte structural integrity that result in unique forms of cardiomyopathy and predisposed susceptibility to death after myocardial stress. These studies further highlight the importance of studying the role of α-E-catenin in human cardiac injury and cardiomyopathy in the future. (Circulation. 2006;114:1046-1055.)

Key Words: genes ■ myocytes ■ structure ■ cardiomyopathy ■ death, sudden

Intercalated discs are highly organized structural components of cardiac muscle that are thought to maintain structural integrity and synchronize contraction of cardiac tissue. Although disorganization of the cardiac intercalated disc has been considered a “hallmark” of cardiac disease,1,2 to date, there is limited information on whether alterations in its structure and/or components play a causal role.

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The intercalated disc is a complex entity that consists of an array of proteins, which are categorized into 3 major junctional complexes: adherens junctions, desmosomes, and gap junctions. Adherens junctions are anchoring junctions between cells that link the cell membrane to the cytoplasmic actin cytoskeleton to provide strong cell adhesion.3 Considerable attention has focused on the adherens junction proteins because human and mouse genetic studies have revealed that mutations/deficiencies in their components are linked to cardiomyopathies and other fatal defects.1,2,4–8 Components of this complex include (1) cadherins, which are transmembrane proteins responsible for Ca2+-dependent homophilic cell-cell adhesion;2 (2) catenins (ie, α-, β-, and γ-catenin), which are cytoplasmic cadherin-binding partners that regulate cadherin adhesive activity9 and are implicated in signaling10; and (3) other catenin-related proteins, including vinculin and α-actinin.3,11–13 The role of cadherins in the postnatal heart was recently established in studies that demonstrated that cardiac-specific loss of N-cadherin resulted in loss of the cardiac intercalated disc, dilated cardiomyopathy, and arrhythmogenic defects in the postnatal heart.14,15 Because all junctional components were lost/reduced in N-cadherin–null hearts,14 however, the mechanism and contribution of the catenins remain to be determined in the myocardium and disease.

Cadherin-mediated cell adhesion is regulated by distinct proteins found within the extracellular and cytoplasmic do-
mains of cadherin.\textsuperscript{3} \(\alpha\)-Catenins are key cytoplasmic molecules that are thought to indispensably link the cytoplasmic domain of cadherin to the actin cytoskeleton.\textsuperscript{16} \(\alpha\)-Catenins are thought to provide this link by binding to \(\beta\)- or \(\gamma\)-catenin through their N-terminal domain and to actin directly through their C-terminus or indirectly by binding to actin-binding proteins, such as vinculin and \(\alpha\)-actinin.\textsuperscript{11} On the basis of recent structural studies, new models have suggested a dynamic as opposed to static role for \(\alpha\)-catenin and vinculin in the cadherin/catenin/actin complex.\textsuperscript{17–19} Three \(\alpha\)-catenin subtypes have been described, which include \(\alpha\)-E-, \(\alpha\)-N- and \(\alpha\)-T catenins.\textsuperscript{17–19} \(\alpha\)-E-catenin is ubiquitously expressed in tissues, mainly in epithelial cells, but also at high levels in the heart.\textsuperscript{1,20} On the other hand, \(\alpha\)-N-catenin expression is restricted to neural tissues,\textsuperscript{18} whereas \(\alpha\)-T-catenin expression is restricted to testis, muscle, and brain.\textsuperscript{20} \(\alpha\)-E-catenin is the most widely studied, and although it is highly expressed in the heart, its functional importance is most notable in tumor cells that have lost functional \(\alpha\)-E-catenin protein, with concomitant loss of cell aggregation and thus gain of invasive capacity.\textsuperscript{21,22} In skin cells, studies have revealed dual functions for \(\alpha\)-E-catenin, not only in adherens junction formation but also in signaling.\textsuperscript{10}

Although \(\alpha\)-E-catenin has been studied primarily in the context of cancer, there is evidence to suggest that it may play a role in cardiac disease. Specifically, \(\alpha\)-E-catenin is highly expressed in the adherens junction of the cardiac intercalated disc, and perturbations in its expression are associated with dilated cardiomyopathy, a disease associated with and shown to influence the cytoskeleton.\textsuperscript{1,2,20,23,24} Because \(\alpha\)-E-catenin is dynamically linked to the cytoskeleton in other cell types, we hypothesized that \(\alpha\)-E-catenin would play an essential role in cardiac adherens junction ultrastructure and function in the myocardium in vivo. \(\alpha\)-Catenin is required for early vertebrate embryonic development\textsuperscript{8} and is ubiquitously expressed; thus, we utilized a conditional approach to explore its functional role in the myocardium. Specifically, we generated cardiomyocyte-specific \(\alpha\)-E-catenin conditional knockout mice (\(\alpha\)-E-cat cKO) using the well-established ventricular form of myosin light chain 2 (MLC2v)–Cre knock-in mice.\textsuperscript{25}

We demonstrate that cardiomyocyte-specific inactivation of \(\alpha\)-E-catenin resulted in a unique form of cardiomyopathy, which included intercalated disc ultrastructural defects and complete loss of vinculin from the intercalated disc. We also demonstrate that \(\alpha\)-E-cat cKO mice are predisposed to ventricular free wall rupture. These findings demonstrate a clear functional role for \(\alpha\)-E-catenin within the cadherin/catenin/vinculin complex in the myocardium in vivo.

**Methods**

**Experimental Animals**

Floxed \(\alpha\)-E-catenin mice (kind gift from Dr Elaine Fuchs, Rockefeller University, New York, NY) have been characterized previously\textsuperscript{10} and were used to generate cardiac-specific \(\alpha\)-E-cat cKO mice, as illustrated in Figure 1A. All animal procedures were in full compliance with the guidelines approved by the University of California—San Diego Animal Care and Use Committee.

**Adult Mouse Cardiomyocyte Isolation**

Please refer to the online-only Data Supplement.

**RNA Blot Analysis**

Total RNA was extracted from cardiomyocytes with Trizol (Invitrogen Corp, Carlsbad, Calif). Total RNA (20 \(\mu\)g) was electrophoresed, blotted, and hybridized with full-length \(\alpha\)-E-catenin cDNA, as described previously.\textsuperscript{26}

**Protein Analysis**

Total protein was prepared from cardiomyocytes, ventricles, and atria as described previously.\textsuperscript{1} Membrane fractions were prepared by layering ventricular extracts onto a linear 5% to 20% sucrose density gradient and subjecting them to ultracentrifugation, fractionation, and concentration as described previously.\textsuperscript{27} Immunodetection of \(\alpha\)-catenin (Sigma-Aldrich, Inc, St Louis, Mo), \(\alpha\)-T catenin (kind gift from Dr Frans van Roy, Ghent University, Ghent, Belgium), \(\beta\)-catenin (Affiniti Research Products Ltd, Plymouth Meeting, Pa), vinculin (Sigma-Aldrich), dystrophin,\textsuperscript{27} \(\alpha\)-catenin (Sigma-Aldrich), desmoplakin (Serotec, Raleigh, NC), plakoglobin (Transduction Laboratories, BD Biosciences, San Jose, Calif), connexin 43 (Chemicon, Temecula, Calif), and all actin (Sigma-Aldrich), was performed as described previously.\textsuperscript{1,28}

**Immunofluorescence Microscopy**

Adult heart cryosections and cardiomyocytes were fixed with 4% paraformaldehyde and stained with primary antibodies against \(\alpha\)-catenin (1:500; Sigma-Aldrich), sarcomeric \(\alpha\)-actinin (1:200; Sigma-Aldrich), \(\beta\)-catenin (1:200; Affiniti Research Products Ltd), and vinculin (1:200; Sigma-Aldrich). Cells were then stained with secondary antibodies (1:250; Molecular Probes, Invitrogen) and Hoechst nuclear stain, followed by visualization of signals by deconvolution imaging (Deltavision, Applied Precision, Inc, Issaquah, Wash).

**Histology**

Transverse sections were obtained at 8-\(\mu\)m intervals and stained with hematoxylin and eosin as described previously.\textsuperscript{28} Masson trichrome (Sigma-Aldrich) and terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (Roche Applied Science, Indianapolis, Ind) staining assays were performed on sections according to the manufacturer’s instructions. DNA fragmentation was verified under ultraviolet illumination with the DAPI nuclear counterstain.

**Echocardiography**

Adult mice were anesthetized with 1% isoflurane and subjected to echocardiography as described previously.\textsuperscript{29}

**Hemodynamic Analysis**

Mice were anesthetized with ketamine/xylazine and ventilated, and a 1.4F Millar catheter-tip micromanometer catheter was inserted through the right jugular vein into the right ventricle of the mouse heart to obtain and record right ventricular pressures, as similarly described for the left ventricle (LV).\textsuperscript{30}

**Electron Microscopy**

Cardiac ventricles were processed for electron microscopy as described previously.\textsuperscript{25,28}

**Myocardial Infarction Model**

A previously established myocardial ischemia/reperfusion protocol\textsuperscript{31} was modified to induce permanent left anterior descending branch ligation in the mouse heart. Quantitative measurement of area at risk, infarct size, and ratio of the infarct area/area at risk was done as described previously.\textsuperscript{31}

**Statistical Analysis**

Data presented in the text and figures are expressed as mean±SEM, unless otherwise stated. Significance has been evaluated by the 2-tailed Student \(t\) test.
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

Generation of α-E-cat cKO Mice
To specifically ablate α-E-catenin in ventricular cardiomyocytes, floxed α/E-catenin mice were bred with MLC2v-Cre knock-in mice, which have been shown to mediate DNA recombination of genes, specifically in ventricular cardiomyocytes. A breeding strategy was used to generate wild-type (WT) and cardiac-restricted α-E-catenin cKO mice, as illustrated in Figure 1A. α-E-catenin cKO mice were born at mendelian ratios and survived until adulthood.

To assess the efficiency of Cre, α-E-catenin expression was assessed in purified cardiomyocytes by RNA and protein analysis at 16 weeks. A significant 80±3.5% (n=3) reduction in α-E-catenin RNA was observed in α-E-catenin cKO purified ventricular cardiomyocytes compared with controls (Figure 1B). A 90±7.0% (n=3) and 90±5.0% (n=3) decrease in α-E-catenin protein expression was observed in purified left and right ventricular cardiomyocytes, respectively, but not in atria of α-E-catenin cKO mouse hearts compared with controls (Figure 1C). Immunofluorescence staining demonstrated that positively stained myocytes in α-E-catenin cKO mice, representative of WT cardiomyocytes, specifically expressed α-E-catenin at the distal polar ends of the cardiomyocytes, which indicates localization to the intercalated disc (Figure 1D). Negatively stained cardiomyocytes within α-E-catenin cKO mice demonstrated absence of α-E-catenin staining at the intercalated disc (Figure 1D).

Progressive Ventricular Cardiomyopathy and Cardiac Dysfunction in α-E-cat cKO Mice
At 32 weeks, α-E-catenin cKO mice revealed modest dilation of the LV and thinning of the right ventricle anterior wall (Figure 2A). At 60 weeks, α-E-catenin cKO mouse hearts showed significant dilation of the LV and thinning of the right ventricle anterior wall compared with controls (Figure 2A). These results were reflected by the significant increase in LV/body weight ratios and the decrease in right ventricle/
body weight ratios in α-E-cat cKO mice at 60 weeks (Figure 3A) compared with controls. We also observed a significant increase in cardiomyocyte apoptosis in ventricles of α-E-cat cKO mouse hearts at 60 weeks (Figure 2B). Interestingly, we did not observe significant fibrosis in the LV of α-E-cat cKO mouse hearts at 60 weeks; however, fibrosis could be detected in the thinned regions of the right ventricle (Figure 2C). No significant differences in cardiomyocyte proliferation, as assessed by phosphorylated histone 3–positive cardiomyocyte nuclei, was detected between α-E-cat cKO and control mouse hearts at 60 weeks (data not shown).

Echocardiography further revealed that α-E-cat cKO mice had significant abnormalities in cardiac dimensions and function, suggestive of dilated cardiomyopathy, at 60 weeks. Figure 3B is a representative echocardiographic M-mode image demonstrating the significant increase in LV internal dimensions in α-E-cat cKO mice compared with control. There were no significant differences in body weight (WT, n=8, 35.3±0.9 g; α-E-cat cKO, n=10, 34.8±1.7 g) or heart rate (WT, n=8, 468±14 bpm; α-E-cat cKO, n=10, 444±18 bpm) between α-E-cat cKO and WT mice. However, α-E-cat cKO mice had significantly increased LV chamber size, as determined by LV end-diastolic dimension and LV end-systolic dimension (Figure 3A). In addition, there was significant wall thinning, as determined by the decrease in systolic dimensions in interventricular septal wall thickness and LV posterior wall thickness, in α-E-cat cKO mouse hearts compared with controls (Figure 3C). Most striking was the presence of reduced LV function, as evidenced by reduced percent fractional shortening in α-E-cat cKO mouse hearts (Figure 3C). Despite functional changes in the LV, there was no evidence of pulmonary edema, as assessed by lung weights in α-E-cat cKO mice (WT, n=5, 0.214±0.02 g; α-E-cat cKO, n=6, 0.214±0.02 g). Hemodynamic analysis of the right ventricle also revealed no significant differences in right ventricular function between α-E-cat cKO mice and controls at 60 weeks of age (see online Data Supplement). No obvious cardiac phenotype was observed in heterozygous floxed α-E-cat cKO mice.

Ultrastructural Abnormalities of the Cardiac Intercalated Disc in α-E-cat cKO Mice

Electron microscopy was performed on α-E-cat cKO mouse hearts to examine the integrity of the intercalated disc at the ultrastructural level. LV and right ventricular regions were obtained from α-E-cat cKO and WT mouse hearts at 32 weeks and assessed for ultrastructural changes in longitudinally oriented myocytes. Severe disorganization in intercalated disc structure was observed in the α-E-cat cKO mouse hearts compared with controls (Figure 4). Cardiac muscle from α-E-cat cKO mice revealed strikingly large, highly convoluted intercalated disc membranes with disorganized/less-aligned mitochondria. In contrast, cardiac muscle from WT mice showed well-aligned/attached arrays of myofibrils at Z-lines and intercalated discs, with visibly aligned mitochondria.
Alterations Within the Adherens Junction Protein Complex in \(\alpha\)-E-caten cKO Mouse Hearts

To determine the influence of \(\alpha\)-E-catenin ablation on the expression of other cardiac intercalated disc proteins, we assessed the expression levels of other adherens junction (pan-cadherin, \(\alpha\)-T-catenin, \(\beta\)-catenin, vinculin, and plakoglobin), desmosome (desmoplakin and plakoglobin), and gap junction (connexin 43) proteins in \(\alpha\)-E-caten cKO and WT mouse ventricles at 20 weeks of age. \(\alpha\)-E-catenin protein levels in \(\alpha\)-E-caten cKO whole ventricles were 35.3 \(\pm\) 8.9% (n = 3; \(P < 0.01\); mean \(\pm\) SD) of control levels (Figure 5), which was comparable to the decrease observed in purified ventricular cardiomyocytes (Figure 1C), because \(\alpha\)-E-catenin is also expressed in noncardiomyocytes. Overall, no significant differences in \(\alpha\)-T-catenin, plakoglobin, desmoplakin, and connexin 43 levels were observed in \(\alpha\)-E-caten cKO ventricles (Figure 5). However, a significant increase in \(\beta\)-catenin levels (163.6 \(\pm\) 35.9% of WT levels; n = 3; \(P < 0.05\)) and decrease in pan-cadherin levels (85.7 \(\pm\) 4.4% of WT levels; n = 3; \(P < 0.05\)) were detected in \(\alpha\)-E-caten cKO ventricles (Figure 5). The most striking difference was the significant reduction in vinculin levels (40 \(\pm\) 5.9% of WT levels; n = 3; \(P < 0.01\)) observed in \(\alpha\)-E-caten cKO ventricles (Figure 5).

Loss of \(\alpha\)-E-Catenin/Vinculin Complex at Cardiac Intercalated Disc of \(\alpha\)-E-Cat cKO Mice

Because vinculin is expressed in various compartments within cardiomyocytes,\textsuperscript{32,33} we determined the subcellular localization of loss of vinculin within \(\alpha\)-E-caten cKO cardiomyocytes. Cardiomyocytes that stained positively for \(\alpha\)-E-catenin in WT and \(\alpha\)-E-caten cKO mice revealed specific
expression of vinculin at the intercalated disc and costameres (green arrows in Figure 6). However, cardiomyocytes lacking α-E-catenin expression at the intercalated disc had loss of vinculin expression at the intercalated disc (Figure 6). The presence of vinculin at the costameres in α-E-catenin cKO myocytes further suggested that the membrane integrity of the α-E-catenin cKO myocyte was not compromised. Unlike vinculin, we demonstrated that β-catenin was overexpressed at the intercalated disc in α-E-catenin cKO cardiomyocytes (Figure 6), which reinforced the increase in expression observed by protein blotting (Figure 5). The same localization of β-catenin was observed in adult cardiomyocytes isolated from WT mice (Figure 6).

To assess the physical integrity of the α-E-catenin/vinculin complex in α-E-catenin cKO mice, membrane fractions from mouse ventricles were separated by sucrose density gradient ultracentrifugation to analyze for the presence of α-E-catenin, β-catenin, vinculin, and dystrophin. In WT mouse ventricles, α-E-catenin, β-catenin, and vinculin cofractionate in fractions 15 to 16 of the gradient (Figure 7), which suggests that they are physically associated. However, in α-E-catenin cKO mouse ventricles, both α-E-catenin and vinculin were lost in fractions 15 to 16 (Figure 7), which suggests physical dissociation of the α-E-catenin/vinculin complex. Because vinculin was still expressed in fractions that were shown not to cofractionate with α-E-catenin, this presumably reflected vinculin expression at other subcellular locations (Figure 7). In addition, β-catenin was still expressed in fractions 15 to 16, where vinculin and α-E-catenin were lost (Figure 7), which demonstrates that the loss of α-E-catenin did not result in loss of expression of other adherens junction proteins in these fractions. The presence of the plasma membrane protein dystrophin in fractions 5 to 8 in both WT and α-E-catenin cKO cardiac membrane fractions further reinforced the efficiency in compartmentalization of different proteins in the sucrose gradient and the specificity of the loss.

α-E-catenin cKO Mice Are Susceptible to Ventricular Wall Rupture

Because plakoglobin (γ-catenin) conventional null mouse hearts exhibited signs of rupture during embryonic development, we hypothesized that α-E-catenin cKO mouse hearts would also have increased vulnerability to cardiac rupture after stress. Cardiac ruptures occur in 1% to 6% of patients with acute myocardial infarction (MI), and therefore, we assessed the effects of MI in α-E-catenin cKO (n = 14) and WT (n = 14) female mice at 20 weeks. No significant differences in early mortality (within 24 hours of surgery) were observed between the 2 groups after MI. In addition, all sham animals of both genotypes survived the surgery and postoperative period up to 7 days after MI. However, 82%, 54%, and 0% of α-E-catenin cKO mice and 100% of WT mice were found to survive at 5, 6, and 7 days after MI, respectively (Figure 8A). Macroscopic assessment of α-E-catenin cKO mice on autopsy revealed the presence of blood in the chest cavity in all mice assessed (Figure 8B), suggestive of death as a result of rupture. In most cases, the site of rupture in α-E-catenin cKO mouse hearts could be identified macroscopically by the visible subepicardial hemorrhage that was localized at border zones of the infarct area and right ventricle of the heart (Figure 8C). α-E-catenin cKO mouse hearts revealed the presence of intramural hematomas (accumulation of multiple hemorrhagic foci; Figure 8D), which is a classic feature of rupture. Because the timing of rupture could not be predicted in α-E-catenin cKO mice, we analyzed infarct size/area at risk in α-E-catenin cKO and WT mice at 4 days after MI, a time point at which all mice survive. No significant differences in infarct size/area at risk could be detected between α-E-catenin cKO (40 ± 4.5%, n = 6) and WT (36 ± 2.9%, n = 6) mice.

Discussion

Recent studies have implicated a role for α-catenins in the postnatal heart. However, because α-catenin plays a vital role in early embryonic development, this has precluded its ability to be studied in the postnatal heart with conventional strategies. We utilized a conditional approach to determine the role of α-E-catenin in cardiomyocytes in vivo. Using

** Figure 5. Alterations in adherens junction protein expression in α-E-catenin cKO mouse whole ventricles.** Top, Protein blot analyses of pan-cadherins, α-E-catenin, α-T-catenin, β-catenin, vinculin, desmoplakin, plakoglobin, and connexin 43 levels in control (n = 3) and α-E-catenin cKO mouse (n = 3) ventricular extracts at 20 weeks of age. All actin levels were assessed in mouse ventricles to normalize for protein loading. Bottom, Densitometry analyses of various intercalated disc proteins in α-E-catenin cKO vs control mice revealed significant changes in pan-cadherin, α-E-catenin, β-catenin, and vinculin. To avoid loading errors, the relative expression level of each protein was first normalized to corresponding actin levels, and then the α-E-catenin cKO results were combined and expressed as a percentage of combined WT results. 100% represented no change between α-E-catenin cKO and WT expression levels. *P < 0.01, **P < 0.05.
MLC2v-Cre mice, we demonstrated the successful ablation of α-E-catenin expression in cardiomyocytes in α-E-cat cKO mice.

Our studies initially suggested that α-E-catenin is dispensable for early cardiac development, because α-E-cat cKO mice survive until adulthood up to 20 weeks with no significant effects on cardiac ultrastructure and function. However, because maximal α-E-catenin protein reduction was not achieved until 16 weeks, we could not rule out the possibility that the lack of a cardiac phenotype at earlier stages of development could be due to incomplete Cre-mediated recombination during development.

Structural alterations in intercalated discs have long been thought to be “hallmarks” of cardiac disease. We now provide evidence from α-E-cat cKO adult mice demonstrating that α-E-catenin is a critical regulator of cardiac intercalated disc structure. Unlike cardiac-specific N-cadherin knockout mice and keratinocyte-specific α-E-catenin knockout mice, in which intercalated discs or adherens junctions do not form, α-E-cat cKO mouse ventricles display enlarged and highly convoluted intercalated disc membranes. These structural defects recapitulate the intercalated disc defects observed in mouse and human models of dilated cardiomyopathy. It has been previously suggested that the increase in membrane convolution between neighboring cardiomyocytes can lead to impaired alignment between myocytes and thus decrease the flexibility/stability/integrity of the contractile tissue, resulting in decreased cardiac output that leads to dilated cardiomyopathy. We have provided morphological and functional evidence that demonstrates that α-E-cat cKO mouse hearts do indeed progress to dilated cardiomyopathy.

The most surprising morphological defect within α-E-cat cKO mice was the appearance of anterior right ventricle wall...
thinning. Despite morphological and ultrastructural defects in α-E-catenin cKO mouse right ventricle, no significant defect in right ventricular function could be detected. It is possible that hemodynamic analyses may lack the sensitivity to detect regional differences in right ventricular function. It is also possible that the severity of the right ventricular defects may not be sufficient to cause changes in overall function, which is supported by the fact that pulmonary congestion, which is a symptom of right heart failure, was not evident in α-E-catenin cKO mice. Right ventricular cardiomyopathies, such as arrhythmogenic right ventricular dysplasia, have been ascribed to defects/mutations in proteins present within the desmosome complex, including desmoplakin and plakoglobin.6,36 However, we could not detect differences in desmoplakin or plakoglobin expression in α-E-catenin cKO mouse ventricles compared with controls. We also did not detect differences in α-E-catenin expression between the right and left adult cardiomyocytes in WT mouse hearts, thereby ruling out the possibility that the endogenous pattern of α-E-catenin expression could account for the right ventricular defects. Therefore, it remains to be determined whether other right ventricle factors or pathways exist that can influence α-E-catenin dynamics.

The most striking molecular defect observed in α-E-catenin cKO mice was the complete loss/dissociation of vinculin at the cardiac intercalated disc. Although structural studies have proposed shared roles for α-catenin and vinculin in regulating actin dynamics,11–13 to date, there is no information on the shared roles of α-catenin and vinculin in the cadherin-catenin complex in vivo. Human genetic studies have implied a role for metavinculin at the intercalated disc and in disease.7,37 Interestingly, the intercalated disc defects found in human patients with metavinculin mutations7 have striking similarities to the intercalated disc defects found within α-E-catenin cKO mouse ventricles. On the basis of our studies, we have provided evidence that vinculin has shared functional roles

Figure 7. α-E-catenin deficiency causes loss of vinculin from the adherens junction enriched fractions. The integrity of the α-E-catenin/vinculin complex within the cardiac membrane fraction was studied with sucrose density gradient ultracentrifugation. A, In WT mouse hearts, α-E-catenin, β-catenin, and vinculin cofractionate in fractions 15 to 16 of the gradient. Vinculin, on the other hand, could be detected in a broad range of fractions.7–16 B, In α-E-catenin cKO mouse hearts, loss of α-E-catenin in fractions 15 to 16 resulted in loss of vinculin in fractions 15 to 16 yet preservation of β-catenin in fractions 15 to 16 of the gradient. Vinculin could still be detected in fractions 6 to 14. Nonfractionated WT mouse cardiac ventricular extracts (H) were used as a positive control to detect endogenous α-E-catenin, β-catenin, vinculin, and dystrophin.

Figure 8. α-E-catenin cKO mice show susceptibility to wall rupture after MI. A, Kaplan-Meier survival curve analysis of WT and α-E-catenin cKO mice up to 7 days after MI. Note the significant death in α-E-catenin cKO mice at 5, 6, and 7 days post-MI. B, Macroscopic appearance of a representative α-E-catenin cKO mouse heart within the mouse at 7 days post-MI, after autopsy. Note the blood in the chest cavity, dilated right ventricle, and most importantly, visible region of rupture (white arrows). C, Microscopic analysis of ruptured region in a representative α-E-catenin cKO mouse heart. Note the visible subepicardial hemorrhage within the ventricle (black arrow). Infarct area is shown by * D, Cardiac sections from α-E-catenin cKO at 7 days after MI were stained for nuclei and cytoplasm with hematoxylin and eosin, respectively. Note the large hematoma present within the ventricular free wall of a representative α-E-catenin cKO mouse heart (black arrow).
with α-E-catenin at the intercalated disc and more specifically, that α-catenin is required for vinculin localization to the cardiac intercalated disc. Because defects in intercalated disc ultrastructure appear after the molecular defects, we believe that the loss of α-E-catenin and concomitant loss of vinculin are critical for the stability of the cardiac intercalated disc in vivo.

The significance of the loss of α-E-catenin/vinculin is more clearly illustrated in MI studies. We showed that α-E-cat cKO adult mice have a striking susceptibility to free wall rupture. Ruptures were not only found at the border zones of the infarct but also within regions of the right ventricle. It is well established that intercalated disc remodeling is observed in the heart after injury.5–8 The results of the present study suggest that the loss of α-E-catenin/vinculin may also be critical in intercalated disc remodeling at the border zone of infarcts. The role of the right ventricle has largely been ignored in MI studies; however, recent studies have revealed that diminished right ventricular function has been observed as a consequence of the ischemic LV.39 As a result, we believe that the stress incurred in the α-E-catenin cKO right ventricle by the MI was sufficient to cause free wall rupture. Because similar infarct sizes were observed in α-E-cat cKO and WT mice, this further reinforced that the molecular disruptions within the adherens junction complex are key factors regulating the structural susceptibility of the ventricle. Heterozygous vinculin mice also exhibit cardiac intercalated disc defects and predisposed susceptibility to death after stress.39 Because we demonstrate specific loss of vinculin at the intercalated disc of α-E-cat cKO myocytes, the present study highlights the critical role of the cadherin-catenin-vinculin complex in cardiac intercalated disc structure/integrity after stress in vivo.

In conclusion, the present study further highlights the importance of studying the role of α-E-catenin in human cardiac injury and cardiomyopathy. There is already some evidence that suggests that α-E-catenin may play an important role in human heart rupture after MI.40 Thus, identification of α-E-catenin mutations within the human population could be of critical importance in predicting populations prone to inherited forms of cardiac disease and/or cardiac rupture.

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Disclosures
None

References
End-to-end connections between myocardial cells, known as intercalated discs, are thought to maintain structural integrity and synchronize contraction of cardiac tissue. Although abnormalities in intercalated disc structure are considered “hallmarks” of cardiomyopathy, to date there is limited information on whether alterations in its structure or components play a causal role. Thus, an improved understanding of the molecular interactions that are important for formation of the “hallmarks” of cardiomyopathy would be of critical importance in predicting populations prone to inherited forms of heart disease.

CLINICAL PERSPECTIVE
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In the article, “α-E-Catenin Inactivation Disrupts the Cardiomyocyte Adherens Junction, Resulting in Cardiomyopathy and Susceptibility to Wall Rupture” by Sheikh et al published in the September 5, 2006, issue (Circulation. 2006;114:1046–1055), the first name of the second author was misspelled. The first name of Yinhong Chen appeared incorrectly as “Yinghong.” The PDF of this article has been corrected. The authors regret this error.

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