Apoptosis Repressor With Caspase Recruitment Domain Is Required for Cardioprotection in Response to Biomechanical and Ischemic Stress

Stefan Donath, MD*; Peifeng Li, PhD*; Christian Willenbockel, DVM; Nidal Al-Saadi, MD; Volkmar Gross, PhD; Thomas Willnow, PhD; Michael Bader, PhD; Ulrich Martin, MD; Johann Bauersachs, MD; Kai C. Wollert, MD; Rainer Dietz, MD; Rüdiger von Harsdorf, MD; on behalf of the German Heart Failure Network

Background—Ischemic heart disease and heart failure are associated with an increased loss of cardiomyocytes due to apoptosis. Whether cardiomyocyte apoptosis plays a causal role in the pathogenesis of heart failure remains enigmatic. The apoptosis repressor with caspase recruitment domain (ARC) is a recently discovered antiapoptotic factor with a highly specific expression pattern in striated muscle and neurons. ARC is a master regulator of cardiac death signaling because it is the only known factor that specifically inhibits both the intrinsic and extrinsic apoptotic death pathway. In this study we attempted to elucidate the physiological role of ARC and to understand pathophysiological consequences resulting from its deletion.

Methods and Results—We generated ARC-deficient mice, which developed normally to adulthood and had no abnormality in cardiac morphogenesis and function under resting conditions. On biomechanical stress induced by aortic banding, ARC-deficient mice developed accelerated cardiomyopathy compared with littermate controls, which was characterized by reduced contractile function, cardiac enlargement, and myocardial fibrosis. Likewise, ischemia/reperfusion injury of ARC-deficient mice resulted in markedly increased myocardial infarct sizes. Although in both instances a significant increase in apoptotic cardiomyocytes could be observed in ARC-deficient mice, neither in vitro nor in vivo studies revealed any effect of ARC on classic hypertrophic cardiomyocyte growth responses. The pathophysiological relevance of downregulated ARC levels was underscored by specimens from failing human hearts showing markedly reduced ARC protein levels.

Conclusions—Our study identifies a tissue-specific antiapoptotic factor that is downregulated in human failing myocardium and that is required for cardioprotection in pressure overload and ischemia. (Circulation. 2006;113:1203-1212.)

Key Words: apoptosis ■ heart failure ■ hypertrophy ■ ischemia ■ myocardial infarction

Heart failure is a leading cause of mortality in the world, responsible for at least 20% of all hospital admissions among persons older than 65 years in the United States.1 Besides multiple mechanisms including desensitization of β-adrenergic receptor signaling, dysregulation of excitation-contraction coupling, alterations in cytoskeletal proteins, and dysfunctional energy utilization implicated in the progressive loss of contractile function in heart failure, controversy exists about the role of apoptosis in heart failure.2 Although it is well illustrated from sections of human failing hearts that myocyte death is occurring at end-stage heart failure.3,4

Clinical Perspective p 1212

whether apoptosis itself plays a causal role in the pathogenesis of heart failure is still unclear.4 Recently, 2 transgenic overexpression models, inducing myocyte apoptosis, provided conclusive proof that enhanced myocyte dropout by itself can cause dilated cardiomyopathy and cardiac failure.5,6 Others proposed apoptosis to be a critical mechanism in the transition from compensatory hypertrophy to heart failure after pressure overload–induced cardiac hypertrophy.7 However, the multiplicity of functions that apply to many factors
and signaling pathways involved in cardiac growth and apoptotic signaling do not allow a clear discrimination of the causing mechanisms. Therefore, the significance of antiapoptotic signaling itself for the cardiac phenotype remains enigmatic.

When the limited capacity of terminally differentiated cardiac myocytes for proliferation is considered, however, it is important to understand how these cell death–promoting and –inhibiting signaling mechanisms are activated in heart disease to establish interventions that can effectively prevent cardiac cell loss and thereby preserve cardiac performance.8

Apoptosis repressor with caspase recruitment domain (ARC) is highly and predominantly expressed in long-lived tissues like heart, skeletal muscle, and brain.9 ARC selectively interacts with the initiator caspases-2 and -8 and thereby attenuates death receptor–induced (CD95, TNFR1, DR3) or adaptor-induced (FADD, TRADD, CLARP) apoptosis.9 Gustafsson et al10 demonstrated that ARC also prevents cytochrome c release and subsequent cell death independently of caspase inhibition by interfering with Bax activation. Inhibition of both the extrinsic and intrinsic death pathways is mediated through nonhomotypic deathfold interactions.11 We have recently shown that the antiapoptotic effect of ARC depends on its phosphorylation at T149 by CK2.12 Notably, under physiological conditions ARC is predominantly phosphorylated, indicating the biological relevance of functional antiapoptotic ARC.12 Hence, the specific interference with both the death receptor and mitochondrial death pathway, as well as its high cardiac expression, makes ARC a unique and central cardiac death repressor. This is supported by the observation that exogenous ARC reduces infarct size after ischemia/reperfusion (I/R) injury of isolated perfused rat hearts and blocks the development of postischemic cardiomyopathy.13,14 Despite several overexpression studies on ARC, little is known about its endogenous function. However, this is of particular significance because exposure of cardiomyocytes to ischemia, hypoxia, or oxidative stress leads to a rapid downregulation of ARC protein levels with subsequent cell death in vitro, suggesting that decreasing ARC levels may function as a critical switch of cardiomyocyte survival.11

In this study we attempted to elucidate the physiological role of ARC and to understand pathophysiological consequences resulting from its deletion in the ARC knockout mouse model.

**Methods**

**Generation and Genotyping of ARC Knockout Mice**

The targeting vector was constructed in pGEM3Zf (+) by insertion of a 1.0-kb DNA fragment containing the 5′ flanking region of the ARC gene, a neomycin resistance cassette, and a 8.0-kb DNA of 3′ flanking DNA. A herpes simplex virus thymidine kinase cassette was positioned adjacent to the short arm for negative selection. The linearized targeting vector underwent electroporation into murine ES cells, and G418-FIAU doubly resistant colonies were picked and screened by polymerase chain reaction (PCR) for the targeted ARC allele. Two positive ES cell clones were injected into blastocysts derived from C57BL/6 mice. Two lines of mice carrying the disrupted ARC allele in the germline were established. The chimeras were bred to C57BL/6 females, and several male mice from both ES cell clones transmitted the disrupted allele to their progeny. Heterozygous (ARC+/−) progeny were identified as carrying the disrupted ARC gene by PCR. The sequences of the wild-type (WT) allele primers were 5′GATACCGAGATCTCTCTAATAAT′ and 5′CAGCGCATCAA GGCTTCGTACTC′. Primers for the disrupted allele were 5′GATACCGAGATCTCTCTAATAAT′ and 5′GATTGGAGAGACATACGAOGCATGC′. ARC+/− mice were interbred to give knockout mice (ARC−/−), which were used for further studies. All experiments were performed on ARC−/− mice and their WT littermates (ARC+/+) and were approved by government authorities.

**Human Heart Samples**

Cardiac specimens are derived from patients who underwent cardiac transplantation for terminal heart failure of ischemic and nonischemic origin. Control samples came from unused donor hearts without evidence of cardiac disease. All human heart samples were snap-frozen from explanted hearts. All procedures were approved by government authorities.

**Histological Examination and Immunohistochemistry**

In brief, hearts were arrested in diastole by injection of 0.15 mL cadmium chloride (100 mmol/L), fixed in 4% paraformaldehyde overnight, and immersed in 30% sucrose for cytoseparation. Sections were stained with hematoxylin-eosin or Masson’s trichrome to detect fibrosis in heart sections. Myocyte cross-sectional area was measured by tracing the outline of 150 to 200 myocytes per 1-μm-thick silver-stained methacrylate section. Activated Bax (1:50 dilution, anti-Bax NT, rabbit polyclonal, Upstate) was detected with the use of a fluorescein isothiocyanate (FITC)–avidin conjugate. Sections were counterstained with the nucleic acid–binding dye DAPI. To distinguish cardiac myocytes from nonmyocyte cell types, we labeled the sections with a primary anti-sarcosomic α-actinin antibody (1:500 dilution; Sigma) and subsequently with a TRITC-conjugated secondary antibody (1:100 dilution; Sigma). Sections were analyzed with a ×40 objective with the use of fluorescence microscopy. Only those nuclei that were labeled with the ligase technique and were identified as cardiac myocytes were included in our quantification of cardiac myocyte apoptosis. Three transverse sections spaced through the heart were analyzed for each animal. Results were confirmed by a nonfluorescent terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay.

**Echocardiographic Assessment**

A 2-dimensional short-axis view of the left ventricle (LV) was obtained with a 12-MHz transducer (Acuson Sequoia, Erlangen, Germany) in anesthetized mice. M-mode tracings were recorded and used to determine LV end-diastolic (LV ED) and LV end-systolic (LV ESV) internal dimensions and interventricular septum thickness in diastole (IVS D) and posterior wall thickness in diastole (P WD). Fractional shortening was calculated by the following equation: [(LV ED−LV ESV)/LV ED]×100. M-mode tracings were recorded and analyzed by a sample-blinded investigator.

**Animal Models of Myocardial Infarction and Pressure Overload**

I/R injury and evaluation of the infarct size were done as described.16 Transverse aortic banding (TAB) and pressure gradient measurements were performed with minor modifications as described.17 In brief, male 8-week-old mice were anesthetized and intubated. Mice were artificially ventilated with a mixture of oxygen and isoflurane...
Specific primers for fetal cardiac genes were designed as published.18 Mix (Qiagen) was performed in triplicate on an iCycler (BioRad). real-time PCR with the use of QuantiTect SYBR Green PCR Master expressed relative to the healthy control.

Transcriptase–PCR reactions gave a single band when analyzed by gel electrophoresis. The specific mRNA content for each gene was normalized to the mRNA content of GAPDH (fetal genes) or 28S (ARC) and quantitated by Southern blot analysis.

To study the biological function of ARC, we disrupted the ARC gene by homologous recombination. A replacement vector was designed that deleted the encoding exons 2, 3, and 4 and replaced them with a neomycin resistance cassette. Thereby, a functional null allele was created for ARC (Figure 1A).

**Quantitative Real-Time PCR**

RNA was isolated from LV apexes with the use of TRIzol reagent (Invitrogen). After DNase I treatment, 0.5 μg RNA was reverse-transcribed with the use of Superscript III (Invitrogen). A hot start real-time PCR with the use of QuantiTect SYBR Green PCR Master Mix (Qiagen) was performed in triplicate on an iCycler (BioRad). Specific primers for fetal cardiac genes were designed as published.18 The sequences of ARC primers were 5′TCTAAAGAGGCTGAAACCG-GAGCC3′ and 5′TCTCAAGAATCCCTGCGATGCT3′. All reverse transcriptase–PCR reactions gave a single band when analyzed by gel electrophoresis. The specific mRNA content for each gene was normalized to the mRNA content of GAPDH (fetal genes) or 28S (ARC) and expressed relative to the healthy control.

**Statistical Analysis**

Results are presented as mean±SEM. Statistical comparisons were performed with 1-way or 2-way ANOVA or ANOVA on ranks with Tukey or Dunn posttest. A probability value of <0.05 was considered significant.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

**Generation and Characterization of ARC Knockout Mice**

To study the biological function of ARC, we disrupted the ARC gene by homologous recombination. A replacement vector was designed that deleted the encoding exons 2, 3, and 4 and replaced them with a neomycin resistance cassette. Thereby, a functional null allele was created for ARC including splice variants Nop30 and Myp (Figure 1A).

**Mice Lacking ARC Are Prone to Cardiac Decompensation After Biomechanical Stress of Pressure Overload**

In the myocardium, chronic ischemic or mechanical stress results in the activation of maladaptive signaling events, leading to cardiomyocyte hypertrophy, myocardial fibrosis, and eventually heart failure.19 To analyze whether ARC contributes to a preservation of the cardiac phenotype during the biomechanical stress response, we used a microsurgical approach to induce cardiac hypertrophy in vivo by pressure overload after TAB.17

At 4 weeks of TAB, myocardial enlargement was more pronounced in ARC−/− mice than in WT littermates (Figure B). ARC−/− mice were interbred to produce ARC−/− mice. Genotypes were determined by a PCR assay and confirmed by Southern blot analysis (Figure 1B and 1C). Mice homozygous for the ARC-null allele were born normally and externally indistinguishable from littermates of other genotypes. ARC−/− mice grew to adulthood without any abnormalities in their general health and appearance. Analysis of lysates from heart, skeletal muscle, and brain confirmed the loss of ARC protein in ARC−/− mice (Figure 1D).

Under resting conditions, there were no significant differences in heart weight, body weight, heart weight/body weight ratio, and single LV cardiomyocyte cross-sectional area of 12-week-old ARC−/− and WT animals (Figures 2B and 3B). ARC−/− hearts showed no cardiac morphological defects, nor did a histological examination with hematoxylin/eosin or Masson’s trichrome staining demonstrate evidence of myofibrillar disarray, necrosis, or ventricular fibrosis (Figure 4B and 4C). Other tissues with abundant ARC expression like skeletal muscle and brain appeared also morphologically and histologically indistinguishable between ARC−/− and WT mice (data not shown). Finally, under normal living conditions, ARC−/− mice displayed neither hypertrophy nor failure of the heart after a 9-month follow-up.
On the basis of LV wall thickness, a comparable degree of LV hypertrophy was seen 2 weeks after TAB in WT and ARC\textsuperscript{+/–} banded hearts (data not shown). However, at 4 weeks after TAB, hearts from ARC\textsuperscript{+/–} mice displayed accelerated LV dilatation compared with only modestly dilated LV of WT banded hearts (Figure 2A and 2D). There was no significant difference between ARC\textsuperscript{+/–} mice and WT controls in the heart weight/body weight ratio after TAB (Figure 2B). However, the lung weight/body weight ratio, an index of pulmonary congestion and LV dysfunction, was significantly elevated in hearts from ARC\textsuperscript{+/–} banded mice compared with unbanded controls and knockouts, whereas hearts from banded WT mice showed no significant change (ARC\textsuperscript{+/–} TAB versus WT sham or ARC\textsuperscript{+/–} sham, \(P<0.05\); Figure 2B). Other fetal genes implicated in hypertrophy such as \(\alpha\)-myosin heavy chain and skeletal actin showed no differential regulation (Figure 2C).

Dilated cardiomyopathy is characterized by the reinduction of a fetal gene profile in the ventricular myocardium.\textsuperscript{20} In this regard, natriuretic peptides are considered highly sensitive markers of cardiac stress and often correlate with the degree of cardiac dysfunction.\textsuperscript{20} Banded hearts from WT mice showed a moderate induction of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) after TAB (by a factor of 11.2 and 2.3, respectively; Figure 2C). In contrast, the gene induction was markedly more pronounced in banded hearts from ARC\textsuperscript{+/–} mice (by a factor of 15.4 and 6.2, respectively), showing statistical significance for BNP (ARC\textsuperscript{+/–} TAB versus WT TAB, \(P<0.01\); Figure 2C).

Other aortic gradients were comparable between banded WT and ARC\textsuperscript{+/–} mice (66±11 mm Hg for WT, 68±10 mm Hg for ARC\textsuperscript{+/–}), fractional shortening measured 4 weeks after TAB was significantly reduced in ARC\textsuperscript{+/–} banded mice compared with WT banded mice (ARC\textsuperscript{+/–} TAB versus WT TAB, \(P<0.01\); Figure 2D). In addition, hearts from ARC\textsuperscript{+/–} mice displayed a significantly greater dilatation than WT mice, as shown by significant increases in LVESSD and LVEDD (ARC\textsuperscript{+/–} TAB versus WT TAB, \(P<0.05\); Figure 2D). Collectively, these findings indicate that ARC is needed to prevent pressure overload–induced cardiomyopathy after pressure overload with signs of overt heart failure in ARC\textsuperscript{+/–} mice.
deterioration of LV remodeling with cardiac dysfunction and overt heart failure.

**ARC Does Not Affect Classic Hypertrophic Growth Responses**

To evaluate whether the decompensation of ARC$^{-/-}$ mice after TAB resulted from an altered cardiac growth response, we analyzed cardiac hypertrophy at the cellular level. Neither the change in myocyte cross-sectional area in hearts from ARC$^{-/-}$ mice at 4 weeks of TAB (108 ± 15 to 210 ± 8 µm² for WT and 99 ± 13 to 223 ± 2 µm² for ARC$^{-/-}$; WT versus ARC$^{-/-}$, $P>0.05$; Figure 3A and 3B) nor single cardiomyocyte length differed from that seen in WT mice (95 ± 1 µm for WT sham, 98 ± 2 µm for ARC$^{-/-}$ sham, 107 ± 3 µm for WT TAB, 111 ± 2 µm for ARC$^{-/-}$ TAB; WT versus ARC$^{-/-}$, $P>0.05$; Figure 3C). Furthermore, neonatal rat cardiomyocytes transfected with AdARC or control virus showed no differences in terms of sarcomere organization, surface area, and protein synthesis in a model of angiotensin II–induced hypertrophy (Figure 3D to 3F). Functional ARC levels were confirmed by an in vitro model of ischemia-induced cardiomyocyte death (Figure 3G).

**Loss of ARC Mediates Progression to Overt Heart Failure by Apoptosis and Myocardial Fibrosis After Mechanical Stress**

It has been reported that LV remodeling is associated with increased cardiac myocyte death.$^{21}$ We examined the effect of ARC ablation on apoptosis induced by TAB (Figure 4A). Two weeks after TAB, the frequency of myocyte apoptosis was significantly increased in ARC$^{-/-}$ banded hearts compared with WT banded hearts (WT, 10.2 ± 2.8; ARC$^{-/-}$, 25.5 ± 3.8; $P<0.01$). The fact that increases in apoptotic myocytes were observed even before the animals manifest signs of heart failure suggests that increases in cardiac myocyte death may contribute at least in part to the deterioration of heart failure.

Acute or chronic loss of cardiac myocytes can result in LV replacement fibrosis. Histological analysis demonstrated marked intermuscular fibrosis in ARC$^{-/-}$ banded hearts compared with banded WT animals (Figure 4B and 4C). These histological findings were supported by a significant increase in the expression level of transforming growth factor-β (TGF-β), a cytokine of major importance in the...
pathogenesis of myocardial fibrosis, in ARC\(^{-/-}\) hearts after TAB (Figure 4D).

Loss of ARC Increases Myocardial Infarctions After I/R Injury

Next, we evaluated the pathophysiological consequences of ARC ablation on infarct size and myocardial viability after ischemic stress. At the conclusion of reperfusion, the sizes of the area at risk and myocardial infarction were quantified with the use of Evans blue and 2,3,5-triphenyltetrazolium chloride staining. Figure 5A depicts representative stained sections from WT and ARC\(^{-/-}\) mice subjected to I/R in vivo. Despite similar regions at risk, ARC\(^{-/-}\) hearts showed a markedly enlarged area of infarction compared with WT hearts (WT, 24.9±2.3%; ARC\(^{-/-}\), 37.8±1.8%; \(P<0.01\)). The mean infarct size of ARC\(^{-/-}\) mice was increased by 52.2% compared with WT animals.

ARC Exerts Cardioprotection by Inhibition of the Mitochondrial Death Pathway During I/R

We investigated whether ARC signaling is important to protect cardiomyocytes from apoptosis and thereby contributes to a smaller infarct size after I/R. Animals of both genotypes subjected to I/R show a typical DNA laddering (Figure 5B). However, DNA ladders of ARC\(^{-/-}\) mice were more intense, indicating a higher amount of apoptosis. Quantification of apoptosis was performed by the in situ DNA ligase method (Figure 5C and 5D). Consistent with the DNA ladder assay, no apoptosis was seen in WT and ARC\(^{-/-}\) mice under basal conditions. In both genotypes I/R was followed by a marked increase of apoptotic cardiomyocytes. Notably, myocardial sections from ARC\(^{-/-}\) mice showed a 2.5-times higher frequency of apoptosis than those of WT animals (WT, 92.5±19.2; ARC\(^{-/-}\), 249.5±46.5; \(P=0.018\)).

During exposure to ischemia and/or reperfusion, cellular fate ultimately depends on mitochondrial signaling. Bax is a crucial mediator of the mitochondrial apoptotic pathway, and its conformational activation is inhibited by several proteins, including ARC.\(^{10,11}\) We assessed activation of Bax using an antibody that preferentially recognizes the active conformation of Bax.\(^{10}\) Consistent with larger myocardial infarctions and increased apoptotic frequencies, immunohistochemistry from ARC\(^{-/-}\) hearts showed a marked activation of Bax compared with WT hearts after I/R (Figure 5E). Thus, endogenous ARC exerts cardioprotection by its antiapoptotic function that is mediated at least in part by inhibition of Bax activation during I/R.

ARC Protein Is Downregulated in End-Stage Human Heart Failure

To obtain a better understanding of the pathophysiological role of ARC in heart disease, protein and mRNA from healthy controls with normal LV function and patients with end-stage heart failure were analyzed for ARC expression. As depicted in Figure 6A and 6B, Western blot analysis demonstrated a 36.7% decrease of ARC protein expression in hearts from human heart failure patients (control, 151.8±3.2 OD; heart failure, 96.3±8.6 OD; \(P=0.012\)). Because of the limited number of heart failure samples, a subdivision in ischemic (n=1), nonischemic (n=4), and unknown (n=1) etiology did not allow a valid subgroup analysis for differences in ARC expression. In contrast, ARC mRNA expression did not differ between healthy and terminal heart failure patients, indicating posttranscriptional regulation (Figure 6C).

Discussion

The results of the present study identify endogenous ARC as a novel cardioprotective factor in vivo. Three major findings from 2 clinically relevant experimental models support this conclusion. First, endogenous ARC ultimately suppresses and thereby decelerates the onset of dilated cardiomyopathy in response to biomechanical stress of
pressure overload. Second, there is a striking 50% increase in infarct size in ARC−/− mice compared with WT littermates after I/R injury. The observed phenotypes in ARC−/− mice did not appear to be secondary to obvious differences in cardiac structure, myocardial histology, coronary artery anatomy, or area at risk between the different groups of animals. Third, biomechanical and ischemic stress lead to an overall increase in the frequency of apoptosis in ARC−/− mice compared with WT mice. Taken together, these observations suggest that ARC plays a crucial role during

---

**Figure 5.** Critical role of endogenous ARC in myocardial infarction due to I/R. A, Evans blue and tetrazolium staining of WT (n=7) and ARC−/− (n=5) hearts. Mice were subjected to 60 minutes of left anterior descending coronary artery occlusion, followed by 24 hours of reperfusion. White areas represent infarced areas. *P<0.01 vs WT. B, Analysis of internucleosomal DNA fragmentation. C, Representative myocardial sections stained by the in situ DNA ligase method. Apoptotic nuclei are shown by the bright green nuclear fluorescence (FITC). Sections were counterstained with anti-sarcomeric α-actinin antibody and DAPI to identify cardiac myocytes and nuclei. D, Quantification of apoptotic cardiac myocytes by the in situ DNA ligase method (n=3 to 4 per group). *P=0.018 vs WT I/R. E, Immunostaining of LV sections against activated Bax. Activated Bax is indicated by the brownish staining (bar=30 μm, representative of n=3).
numbers of apoptotic cardiac nuclei in hearts from ARC banded WT mice.4 Furthermore, Wencker et al5 reported that apoptotic rates of 23 cardiac nuclei per 10^5 nuclei are sufficient to induce lethal, dilated cardiomyopathy. The fact that increases in apoptotic myocytes were observed even before the animals manifest overt signs of heart failure implies that augmented cardiomyocyte death mediated the progression to cardiac dysfunction and failure seen in ARC^-/-mice. This hypothesis is supported by studies that document progressive cardiomyocyte apoptosis before the development of adaptive hypertrophy in early pressure overload.22,23 Two other studies linked cardiomyocyte apoptosis to the development of decompensated heart failure.7,24 Biomechanical stress of gp130 knockouts resulted in rapid-onset dilated cardiomyopathy, and female mice overexpressing Gaq developed lethal, dilated cardiomyopathy within 1 week after delivery.7,24 Because both gp130- and Gaq-dependent signaling also affects cardiac growth pathways, the role of cardiomyocyte apoptosis in heart failure has been a matter of debate.25,26 The results of the present study shed new light on this discussion. Although we cannot fully exclude the possibility that ARC may affect adaptive growth responses in the heart, according to our in vivo and in vitro results, ARC appears to have no effect on classic cardiac hypertrophic signaling. The combined effects on compensatory growth mechanisms and antiapoptotic signaling may explain the severe and lethal cardiomyopathies observed with the gp130- and Gaq- manipulated mice compared with the rather moderate heart failure phenotype of the ARC^-/- mouse. Our results indicate that loss of antiapoptotic signaling itself can result in progressive cardiac myocyte apoptosis, which in turn leads to accelerated ventricular dilatation, replacement fibrosis, and failure in response to pressure overload. We can only speculate on the mechanism by which increased susceptibility to apoptosis leads to increased expression of natriuretic peptides. As stated in the review by Dorn et al,20 natriuretic peptides such as ANP and BNP do not necessarily represent hypertrophy markers but rather are markers of cardiac dysfunction. We hypothesize that increased wall stress resulting from enhanced apoptosis and ventricular dilatation in ARC-null mice after TAB might lead to forced transcription of natriuretic peptides. Importantly, not all cells showing apoptotic changes die immediately. Evidence was found that during the apoptotic process deterioration in systolic function may precede any breakdown of DNA or irreversible cell death.27 This phenomenon could explain upregulated cardiac stress markers in myocardial tissues with increased susceptibility to apoptosis.

I/R-induced cardiomyocyte apoptosis has been shown in both human heart disease and experimental animal models.3,28,29 The experimental findings here show a correlation between an increase in the frequency of apoptosis and the extent of myocardial infarct size. Accordingly, we speculate that in our model, enhanced cardiac myocyte apoptosis contributed significantly to markedly increased infarct size in ARC knockout mice compared with infarcts of WT control mice. Quantification of apoptosis and oncosis 2 hours after ongoing ischemia by Anversa et al29 yielded 30-fold higher levels of apoptosis. Reperfusion greatly enhances the occurrence of apoptosis.28 Ultimately, cellular fate depends on mitochondrial signaling during exposure to ischemia and/or
reperfusion. Depending on conditions, mitochondria promote both apoptosis by the mitochondrial death pathway and oncrosis by irreversible damage to mitochondria in association with mitochondrial permeability transition. Sustained Bax activation in ARCl-depleted mice detected after reperfusion suggests the activation of the mitochondrial death pathway. Bax plays a critical role in I/R injury, as illustrated by a 50% reduction in infarct size and a 10-fold decrease in myocyte apoptosis exhibited by hearts from Bax knockout mice. 

Unexpectedly, but similar to the results from knockout mice of other ubiquitously expressed antiapoptotic factors, ARCl-null mice developed no basal cardiac phenotype during 9 months of follow-up. Furthermore, gross behavioral observations and histological analyses of sections from brain and skeletal muscle yielded no significant differences between both genotypes. This suggests that either ARCl plays no role under resting conditions or loss of ARCl is compensated by other mechanisms, which may suffice for basal but not for stress conditions. Thus, substantially reduced ARCl protein levels in human failing hearts may be an initiating event rather than a consequence of the resulting cell death. Despite the fact that both gene-targeted animal and cell culture models have obvious limitations, it is tempting to speculate that reduced ARCl protein levels detected in end-stage heart failure patients might represent a causal component of pathogenesis. Further investigation will be necessary for a precise molecular characterization of the role of ARCl during the development of heart failure. Future studies will also have to test the role of the splice variants of ARCl on growth signaling and apoptosis in more detail.

The critical role of endogenous ARCl during biomechanical and ischemic stress provides a strong rationale for therapeutic interventions that focus on the central death pathways. The molecular characterization of the role of ARCl during the development of heart failure and apoptosis in more detail.

Acknowledgments
This work was supported by grants from the Deutsche Forschungsgemeinschaft (Ha 1777/7–4, to Dr von Harsdorff) and the Bundesministerium für Bildung und Forschung (German Heart Failure Network, to Drs von Harsdorff and Wollert). We thank Janet Lips, Daniela Grothe, Marc Eigen, Kai Hu, Charlotte Dienesch, and Helga Wagner for excellent technical assistance.

Disclosures
None.

References
The prevalence of heart failure has increased over the past decades mainly as a result of the reduction of mortality after acute myocardial infarction. Despite an improvement in outcome with standard medical treatment, the prognosis for patients with heart failure still remains poor. Therefore, there is a great need for a better understanding of the molecular basis of heart failure, which could allow us to develop more specific and even more effective therapies. Because the failing heart is characterized by progressive thinning of the ventricular wall, persistent cardiomyocyte death was speculated to contribute to its phenotype. In contrast to necrosis, apoptosis is a highly regulated energy-consuming cell death process with the potential to be antagonized. The search for cardiac apoptosis-related molecules that could be used as therapeutic targets has been hampered by the fact that because of its inevitable nature for organ function and development, apoptosis occurs in every mammalian organ and as such requires ubiquitously expressed protein mediators. In this regard, ARC, a recently identified apoptosis repressor, has become a research focus because of its expression pattern limited to terminally differentiated cells including neurons and skeletal and cardiac muscle cells. In this study we provide evidence for the protective role of antiapoptotic ARC in heart failure. Using biomechanical and ischemic heart failure models in ARC-deficient mice, we proved the hypothesis that apoptosis is causally related to heart failure and that ARC exerts a protective effect in the heart. Our demonstration that ARC protein levels are significantly reduced in human end-stage heart failure makes this molecule an interesting target for future therapies.
Apoptosis Repressor With Caspase Recruitment Domain Is Required for Cardioprotection in Response to Biomechanical and Ischemic Stress

Stefan Donath, Peifeng Li, Christian Willenbockel, Nidal Al-Saadi, Volkmar Gross, Thomas Willnow, Michael Bader, Ulrich Martin, Johann Bauersachs, Kai C. Wollert, Rainer Dietz and Rüdiger von Harsdorf

on behalf of the German Heart Failure Network

_Circulation_. 2006;113:1203-1212; originally published online February 27, 2006;
doi: 10.1161/CIRCULATIONAHA.105.576785

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/113/9/1203