Survivin Determines Cardiac Function by Controlling Total Cardiomyocyte Number

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Background—Survivin inhibits apoptosis and regulates cell division in many organs, but its function in the heart is unknown.

Methods and Results—We show that cardiac-specific deletion of survivin resulted in premature cardiac death. The underlying cause was a dramatic reduction in total cardiomyocyte numbers as determined by a stereological method for quantification of cells per organ. The resulting increased hemodynamic load per cell led to progressive heart failure as assessed by echocardiography, magnetic resonance imaging, positron emission tomography, and invasive catheterization. The reduction in total cardiomyocyte number in α-myosin heavy chain (MHC)–survivin−/− mice was due to an ≈50% lower mitotic rate without increased apoptosis. This occurred at the expense of DNA accumulation because survivin-deficient cardiomyocytes displayed marked DNA polyploidy indicative of consecutive rounds of DNA replication without cell division. Survivin small interfering RNA knockdown in neonatal rat cardiomyocytes also led to polyploidization and cell cycle arrest without apoptosis. Adenoviral overexpression of survivin in cardiomyocytes inhibited doxorubicin-induced apoptosis, induced DNA synthesis, and promoted cell cycle progression. The phenotype of the αMHC-survivin−/− mice also allowed us to determine the minimum cardiomyocyte number sufficient for normal cardiac function. In human cardiomyopathy, survivin was potently induced in the failing heart and downregulated again after hemodynamic support by a left ventricular assist device. Its expression positively correlated with the mean cardiomyocyte DNA content.

Conclusions—We suggest that the ontogenetically determined cardiomyocyte number may be an independent factor in the susceptibility to cardiac diseases. Through its profound impact on both cardiomyocyte replication and apoptosis, survivin may emerge as a promising new target for myocardial regeneration. (Circulation. 2008;117:1583-1593.)

Key Words: apoptosis ■ cardiomyopathy ■ heart-assist device ■ heart failure ■ myocardium ■ physiology ■ transplantation

Heart failure is associated with a high mortality rate and poor quality of life. Its incidence is rapidly increasing, accounting for 20% of all hospital admissions in individuals >65 years of age in the United States.1 Apoptosis of cardiomyocytes leading to loss of contractile units has been implicated in the pathogenesis of heart failure. Although the exact stimuli, mechanisms, and rate of apoptosis in the adult human heart are unknown, a dynamic balance exists between cardiomyocyte loss and replacement that controls cardiac fate during life and disease.2

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Caspases, the common executioners of the apoptotic program, are normally held in check by the inhibitor of apoptosis...
Survivin is a member of the inhibitor of apoptosis protein family with a unique structure devoid of the caspase-binding region present in all other inhibitor of apoptosis proteins. Nevertheless, it efficiently inhibits mitochondrial apoptosis by inhibiting caspases. In addition, survivin regulates caspase-independent cell death and mitochondrial membrane permeabilization. Apart from its role in cell death, survivin has an important function in cell division: It controls multiple phases of mitosis by regulating the spindle assembly checkpoint, microtubule stability, metaphase spindle formation, and the chromosomal passenger proteins aurora B kinase and INCENP. Accordingly, survivin is ubiquitously expressed during development and in a variety of human malignancies. The unfavorable outcome associated with high survivin expression in clinical outcomes with an image analysis program (KS 300, Zeiss, Germany). To identify unique roles of survivin in cardiomyocytes, we provide evidence that survivin controls cardiomyocyte proliferation and ploidy and that its loss leads to progressive heart failure in cardiomyocyte-specific survivin-deficient mice, we provide evidence that survivin controls cardiomyocyte proliferation and ploidy and that its loss leads to progressive heart failure through a reduction in total cardiomyocyte numbers. In addition, we identify unique roles of survivin in cardiomyocyte DNA content, proliferation, and death.

Methods
A detailed Material and Methods section is available in the online-only Data Supplement.

Generation of Mice With Cardiac-Specific Deletion of Survivin
Mice homozygous for a floxed survivin allele were crossed with heterozygous mice that express Cre recombinase under the control of the α-myosin heavy chain (αMHC-Cre). For genotyping of microdissected cardiomyocytes, deletion-specific polymerase chain reaction (PCR) was performed. For single-cell laser microdissection, a PALM Robot-Microbeam (PALM GmbH, Bernried, Germany) was applied, and 60 individual cardiomyocytes or interstitial cells were separately dissected and pooled.

Mean Cardiomyocyte Diameter and Calculation of Total Cardiomyocyte Number per Heart
The mean cardiomyocyte diameter and length were determined by measuring 100 cardiomyocytes on periodic acid-Schiff-stained sections with an image analysis program (KS 300, Zeiss, Germany). To calculate the absolute number of cardiomyocytes per heart, an established 3-dimensional stereological method was used. Briefly, the volume fraction of cardiomyocytes (Vv Myo) in 10 randomly selected visual fields was determined by the principle of Delesse (area density×volume density). A grid containing 513 points was laid over the images, and the points encountering cardiomyocytes, blood vessels, and connective tissue were counted. Vv Myo was calculated as follows: Vv Myo=(V Myo×LV volume)/V Myo, where the total tissue volume of the left ventricle (LV volume) was obtained by dividing its weight by specific gravity (1.0048) and the cell number is given in 10⁶. In embryo studies, the whole embryos were embedded and serial sections were performed parallel to the longitudinal axis, yielding ~60 sections. Cardiomyocytes were counted on every fifth slide containing cardiac tissue.

Immunohistochemistry, Terminal Deoxynucleotidyl Transferase–Mediated dUTP Nick-End Labeling, Fibrosis, and Measurement of DNA Content
Immunohistochemistry for survivin was performed with polyclonal (Acris, Hiddendenhausen, Germany) and monoclonal (60.11, Novus, Littleton, Colo) antibodies for mouse and human tissue, respectively. Terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) was performed with ApopTag Plus (Oncor, Gaithersburg, Md) and FITC–anti-digoxigenin (Roche, Mannheim, Germany). The percentage of TUNEL-positive nuclei was calculated in 10 visual fields. Fibrosis (mean percentage of area) was determined with Sirius Red. DNA content was determined by automated DNA cytometry. Briefly, the Feulgen reaction was applied to nuclei isolated from three 50-μm paraffin sections after hydrolysis, stained with Schiff’s reagent, and analyzed with DNA cytometry software (CYDOK, Fa. Hilgers, Königswinter, Germany).

Magnetic Resonance Imaging, Positron Emission Tomography, Echocardiography, and In Vivo Hemodynamic Measurements
Magnetic resonance imaging (MRI) was performed with a Bruker DRX 9.4-T wide-bore nuclear magnetic resonance spectrometer as described. Positron emission tomography (PET) was performed with [18F]FDG and a small animal camera (quadHIDAC, Oxford Positron, Oxford, England). High-resolution echocardiography was performed with an ultrasound device with frame rates up to 280 Hz (Philips Medical Systems, Bothell, Wash). Left ventricular catheterization was performed in closed-chest mice as described. Increasing doses of dobutamine were perfused into the left jugular vein, accompanied by measurements of heart rate, maximal left ventricular pressure, and the first derivative of left ventricular pressure.

Cardiomyocyte Culture, Small Interfering RNA Transfection, and Adenoviral Infection
Rat neonatal cardiomyocytes were infected with adenovirus carrying survivin or green fluorescent protein (GFP) for 24 hours as described. Apoptosis was induced with 1 μmol/L doxorubicin, and DNA fragmentation was examined by flow cytometry. [3H]thymidine incorporation and small interfering RNA (siRNA) transfection were performed as described. Cell cycle profiles were analyzed in an EpicsXL flow cytometer (Beckman Coulter, Fullerton, Calif).

Studies in Patients With Heart Failure and Left Ventricular Assist Device Support
Ten male patients underwent left ventricular assist device (LVAD) implantation for therapy of end-stage chronic heart failure as a bridge to transplantation (7 patients received a Novacor N100 [Baxter Healthcare Corp, Novacor, Oakland, Calif]. 3 patients received a DeBakey/NASA device [MicroMed Cardiovascular Inc, Houston, Tex]). Four patients suffered from dilated cardiomyopathy and 6 from ischemic heart disease. The mean duration of LVAD support was 129.9 days (median, 76 days; range, 17 to 298 days). Five donor hearts served as controls. The numerical density of positive cells per visual field of defined size was determined following the rules of the forbidden and permitted lines in subepicardial, midendocardial, and subendocardial areas in 7 randomly selected visual fields in a blinded fashion. The present study was performed according to the Declaration of Helsinki. Written consent was obtained from each patient.
Figure 1. Generation of cardiomyocyte-specific survivin-deficient mice. A, Ablation of survivin in cardiomyocytes of αMHC-survivin−/− mice is confirmed by single-cell PCR of laser-microdissected cardiomyocytes (top) and interstitial cells (bottom). Nuclei were labeled for excision (red circles), and PCR was performed for floxed and deleted survivin (500-bp product when the allele was intact, 425-bp product when excised). PCR also was performed for Cre (lane 3). B, Heart pathology of a 30-month-old αMHC-survivin−/− mouse and a wild-type littermate control. Echocardiography shows long-axis view with a large thrombus (*) in the right atrium and tricuspid regurgitation with an enlarged pulmonary trunk (black arrow, right ventricular inflow; black arrowhead, LV inflow). Note the massive enlargement and dilation of both ventricles and atria with fresh and old organized thrombi. C, D, Representative MRI and 18F-FDG-PET images of a 20-week-old αMHC-survivin−/− mouse and a wild-type control.

Statistical Analysis
All data are expressed as mean±SEM and depicted as box plots when appropriate. Student’s t test was used to evaluate statistical significance between the different genotypes for all hemodynamic and morphometry data, including heart weight. Statistical significance between different time points was determined by use of 1-way ANOVA followed by multiple-comparison procedures according to Duncan. Overall survival curves were estimated by the Kaplan–Meier method, and differences between genotypes were compared by the log-rank test. The nonparametric Wilcoxon test for paired samples was used to evaluate statistical significance for survivin immunoreactivity in the myocardium before and after LVAD support. Intergroup differences among samples before and after LVAD and controls were calculated by 1-way ANOVA followed by post-hoc analysis according to Duncan. Correlation analysis was performed according to Spearman. A value of P<0.05 was considered significant.

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

Results
Progressive Heart Failure and Death in αMHC-Survivin−/− Mice
Mice deficient for cardiac survivin were generated by crossing survivin flox/flox mice with mice expressing Cre recombinase under the cardiac-specific αMHC promoter. Laser capture microdissection and single-cell PCR (Figure 1A) and immunohistochemistry (Figure 5) revealed that survivin has been exclusively deleted in cardiomyocytes and not in cardiac fibroblasts of αMHC-survivin−/− mice. Although survivin-deficient mice were born with the expected mendelian frequency and were indistinguishable from their Cre-negative littermates at birth, they died prematurely with a median survival of 34 weeks (Figure 2A). Before death, αMHC-survivin−/− mice developed a characteristic syndrome consisting of decreased activity, tachypnea, hunched posture, and poor grooming. Serial echocardiography uncovered massive enlargement of all cardiac cavities, pericardial effusions, and atrial thrombi (Figure 1B). On autopsy, all chambers of the heart were enlarged (Figure 1B), and pathological findings consistent with decompensated heart failure such as pericardial and pleural effusions, ascites, congested lungs, and an enlarged liver were present (data not shown). MRI confirmed the profound functional and structural cardiac abnormalities of αMHC-survivin−/− mice (Figure 1C and 1D). These abnormalities were not due to regional myocardial viability defects as excluded by [18F]FDG-PET (Figure 1C and 1D).

Quantitative analysis of cardiac function by MRI revealed a progressive impairment of cardiac function in αMHC-survivin−/− mice at 18 and 36 weeks (Figure 2B). In particular, end-diastolic and end-systolic volumes were significantly higher in αMHC-survivin−/− mice compared with controls and increased progressively in an age-dependent manner (Figure 2B). Whereas stroke volume and cardiac output were still maintained in 18- and 36-week-old αMHC-
survivin \(^{-/-}\) mice, the mean ejection fraction was decreased by 17% and 29%, respectively, compared with controls, and fractional shortening and wall thickening were progressively reduced (Table). Invasive cardiac catheterization performed to assess basal and dobutamine-stimulated left ventricular pressures in vivo revealed that in αMHC-survivin \(^{-/-}\) mice maximal left ventricular pressure and contraction/relaxation rates were already lower under basal conditions compared with controls (Figure 2C through 2F). When stimulated with increasing doses of dobutamine, survivin-deficient hearts developed higher heart rates, lower left ventricular pressures, and lower maximum rates of contraction/relaxation compared with controls (Figure 2C through 2F). When stimulated with increasing doses of dobutamine, survivin-deficient hearts developed higher heart rates, lower left ventricular pressures, and lower maximum rates of contraction/relaxation compared with controls (Figure 2C through 2F).

**Figure 2.** Functional characteristics. A, Survival analysis of 30 αMHC-survivin \(^{-/-}\) and 35 wild-type mice. B, End-diastolic (EDV) and end-systolic (ESV) volumes of 18- and 36-week-old αMHC-survivin \(^{-/-}\) mice and controls as assessed by MRI (\(P<0.05\) vs wild type, \(P<0.05\) between 18 and 36 weeks, Student’s t test). C, Heart rate; E, left ventricular pressure. D and F, Maximum (Max.) rate of contraction and relaxation, respectively, under basal conditions and in response to increasing dobutamine doses in 28-week-old αMHC-survivin \(^{-/-}\) mice and controls as assessed by invasive hemodynamic measurements (\(P<0.05\), Student’s t test).

However, the most striking morphological observation was the marked enlargement of cardiomyocyte nuclei, along with massive invaginations of the nuclear envelope in survivin-deficient hearts (Figure 3D). The appearance of such gigantic nuclei prompted us to determine whether they contained higher amounts of DNA. Using automated DNA cytometry, we calculated that survivin-deficient cardiomyocytes exhibited an accentuated polyploidy: \(\approx70\%\) of the cells displayed a DNA content of \(\geq4n\) with a few individual cells reaching even \(16n\) (Figure 3E). The mean cardiomyocyte DNA content in αMHC-survivin \(^{-/-}\) mice was \(\approx2\)-fold higher compared with controls at all ages examined (Figure 3F).

One possibility for the accumulation of polyploid DNA may be the occurrence of DNA duplication without consecutive cell division. Thus, we looked for signs of its ultimate consequence: the presence of a net reduction in total cardiomyocyte numbers per heart. Using an established 3-dimensional stereological approach,\(^{15,16}\) we compared the total cardiomyocyte number per heart in control and αMHC-survivin \(^{-/-}\) mice. Strikingly, αMHC-survivin \(^{-/-}\) mice had 34% fewer cardiomyocytes per heart than controls already at birth (Figure 4A). This difference became even more pronounced during the following 4 weeks (\(\approx60\%\) fewer cardiomyocytes in survivin-deficient hearts; Figure 4A). In addition, although the total cardiomyocyte numbers in control

**Polyploidy and Reduction in Total Cardiomyocyte Numbers in αMHC-Survivin \(^{-/-}\) Mice**

The relative heart weights of αMHC-survivin \(^{-/-}\) and control mice were similar at any age examined (Figure 3A). However, there was a clear difference in mean cardiomyocyte diameter in αMHC-survivin \(^{-/-}\) mice compared with controls already at birth; this difference increased progressively with age (from 13% higher diameters at birth to 80% at 270 days; Figure 3B). There was also a 2- to 3-fold increase in interstitial fibrosis with a reticular pattern and subendocardial accentuation in survivin-deficient hearts beginning at 28 days (Figure 3C).

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mice continued to increase during the first 4 weeks after birth, the number of survivin-deficient cardiomyocytes did not (Figure 4A). There was an inverse correlation between cardiomyocyte size and total cardiomyocyte number per heart as reflected by an exponential increase in cell volume with decreasing total cardiomyocyte number (which was striking below an apparent threshold of $\approx 5 \times 10^6$ cardiomyocytes per heart; Figure 4B).

To identify the cause of the reduction in total cardiomyocyte numbers in αMHC-survivin$^{-/-}$ mice, we tested whether there were alterations in the 2 major mechanisms that govern the cellularity of an organ: cell death and cell division. Cardiomyocyte apoptosis was extremely low in both genotypes without any differences as measured by TUNEL assays at all ages (Figure 4C). However, there were clear differences in the number of dividing cardiomyocytes in newborn mice; there was an $\approx 50\%$ reduction in the number of mitotic figures in cardiomyocytes from αMHC-survivin$^{-/-}$ mice compared with controls, together with evidence of aberrant mitosis (Figure 4D). Pronounced nuclear abnormalities such as huge and irregularly shaped nuclei were already present in survivin-deficient cardiomyocytes at embryonic day 10.5, along with the loss of survivin immunostaining (Figure 5), suggesting that at this development stage, successful deletion of the gene had already occurred and had led to defects in cell division (Figure 5).

### Effects of Survivin Knockdown and Overexpression on Ploidy, Apoptosis, DNA Synthesis, and Cell Cycle in Neonatal Cardiomyocytes

To address the biological mechanism behind the αMHC-survivin$^{-/-}$ phenotype in vitro, we knocked down survivin by siRNA and overexpressed it by adenoviral infection in rat neonatal cardiomyocytes. Survivin siRNA induced cell cycle arrest in G2/M with 60% and 71% more cells accumulating in the G2/M phase of the cell cycle after 48 and 72 hours, respectively ($P<0.05$; Figure 6A). Knockdown of survivin also had profound effects on nuclear morphology as evidenced by the appearance of large and bizarrely shaped nuclei (Figure 6A). These nuclei strongly resembled those of cardiomyocytes from αMHC-survivin$^{-/-}$ mice (Figure 3D and 5).

To test for effects of survivin overexpression, we infected rat neonatal cardiomyocytes with adenovirus encoding the survivin gene or a control virus carrying GFP (Figure 6C). Overexpression of survivin induced DNA synthesis, and cell cycle arrest in G2/M with 60% and 71% more cells accumulating in G2/M phase of the cell cycle after 48 and 72 hours, respectively ($P<0.05$; Figure 6A). Knockdown of survivin also had profound effects on nuclear morphology as evidenced by the appearance of large and bizarrely shaped nuclei (Figure 6A). These nuclei strongly resembled those of cardiomyocytes from αMHC-survivin$^{-/-}$ mice (Figure 3D and 5).

To test for effects of survivin overexpression, we infected rat neonatal cardiomyocytes with adenovirus encoding the survivin gene or a control virus carrying GFP (Figure 6C). Survivin overexpression markedly protected cardiomyocytes against doxorubicin-induced apoptosis compared with GFP-infected cells (Figure 6C). Furthermore, it induced DNA
Figure 3. Morphological characterization of αMHC-survivin−/− mice. A, Relative heart weights (heart weight/body weight). B, Cardiomyocyte diameters. C, Cardiac fibrosis in mice at different ages. D, Representative hematoxylin-eosin staining and electron microscopy of an 18-week-old αMHC-survivin−/− mouse and a littermate control. The massive enlargement and bizarre nuclear morphology in cardiomyocytes from αMHC-survivin−/− hearts are marked by arrows and shown in the 2 insets. Bars indicate diameter of individual cardiomyocytes. Electron microscopy depicts distinct nuclear enlargement (N) and bizarre nuclear membrane invaginations in αMHC-survivin−/− cardiomyocytes. E, Representative DNA cytometry of a 28-day-old αMHC-survivin−/− mouse and a littermate control shows distribution of diploid (2n), tetraploid (4n), or polyploid cardiomyocytes. Cardiac fibroblasts were used as reference cells for 2n. F, Total DNA content of knockout and control mice at different ages. Black box depicts the median. *Significant differences vs wild type.
synthesis as measured by [3H]thymidine incorporation and promoted cell cycle progression as evidenced by the increased number of cells in the G2/M phase of the cell cycle (Figure 6C).

Survivin Is Upregulated in the Failing Human Heart and Decreases Again After Hemodynamic Support With a LVAD

Survivin was detectable at extremely very low levels in the normal human heart, but its expression was dramatically increased in the hearts of patients with terminal heart failure resulting from ischemic or dilative cardiomyopathy (Figure 7). Failing hearts showed ~8-fold more survivin-positive cardiomyocytes than donor hearts used as control (median, 3.3; range, 1.8 to 4.7 versus median, 0.4; range, 0.0 to 0.9; Figure 7). Remarkably, hemodynamic support through an LVAD (for the average of 130 days) resulted in a distinct ~60% decrease in the number of survivin-expressing cardiomyocytes when matched samples of identical hearts were examined (median, 3.3; range, 1.8 to 4.7 versus median, 1.4; range, 0.7 to 5.1; P<0.007, Wilcoxon test for paired samples; Figure 7). This suggests that survivin is reversibly regulated by the hemodynamic load affecting the failing heart. Furthermore, survivin expression correlated significantly with the mean DNA content in all examined hearts, including control, failing, and supported hearts (R=0.48, P<0.05, correlation according to Spearman; Figure 8).

Discussion

Survivin is a key regulator of mitotic progression, and its inactivation in mammalian cells or that of its orthologues in lower organisms causes severe mitotic defects.8 Deletion of survivin has been shown to be embryonically lethal with 3 characteristic abnormalities: macronucleation and multinucleation with polyploidization, reduced cellularity, and increased apoptosis.23 In our study, we have observed 2 of these features, marked polyploidy and reduced cell numbers, in vivo and in vitro but found no evidence of enhanced apoptosis. In fact, it has been shown that apoptosis must not necessarily follow survivin suppression despite the occurrence of pronounced cell division defects.4,7 Some cell types fail to arrest after survivin suppression,24 whereas others exit
mitosis to reform single tetraploid nuclei\textsuperscript{23}; in both cases, apoptosis does not take place.

In our study, the marked DNA polyploidization and G2/M arrest in vivo and in vitro suggest that survivin is necessary for regular cardiomyocyte proliferation and mitosis. However, they also suggest that its loss is not sufficient to completely abrogate DNA replication in vivo because a subpopulation of cells exhibited an 8n and even a 16n DNA

![Immunostaining for survivin in αMHC-survivin\textsuperscript{-/-} and control hearts at embryonic day 10.5. Note the enlarged, bizarrely shaped cardiomyocyte nuclei devoid of survivin immunostaining in αMHC-survivin\textsuperscript{-/-} mice.](image)

**Figure 5.** Immunostaining for survivin in αMHC-survivin\textsuperscript{-/-} and control hearts at embryonic day 10.5. Note the enlarged, bizarrely shaped cardiomyocyte nuclei devoid of survivin immunostaining in αMHC-survivin\textsuperscript{-/-} mice.

![Cardiomyocyte cell cycle profile](image)

**Figure 6.** Effect of survivin siRNA knockdown and adenoviral overexpression on cell cycle, proliferation, and apoptosis in rat neonatal cardiomyocytes. A, DNA profiles of cardiomyocytes transfected with siRNA against survivin and nonsilencing siRNA at different times after transfection. Parallel cultures were immunostained for desmin, and nuclei were counterstained with DAPI 72 hours after transfection. B, Survivin overexpression prevents apoptosis (top) and induces DNA synthesis (bottom). DNA fragmentation was quantified by flow cytometry after 24 hours of doxorubicin (Dox) treatment (\*p<0.05, Student’s t test). DNA synthesis was measured by \[^{[3]H}\] thymidine after adenoviral overexpression of survivin (Ad-surv) or GFP (Ad-GFP). Fetal bovine serum (FBS) was used as a control. Western blot analysis shows expression of survivin in infected cardiomyocytes (viral particles [VP]). C, DNA profiles of cardiomyocytes adenovirally overexpressing survivin and their respective GFP controls 48 hours after adenoviral infection.
content, indicative of several consecutive rounds of DNA replication without cell division. The missing increase in apoptosis in survivin-deficient hearts indicates that this polyploidization had not activated any of the cell cycle checkpoints that normally lead to apoptosis in the case of abnormal DNA content. The data also suggest that survivin is necessary for the actual cardiomyocyte division that follows DNA replication because deletion of survivin resulted in a dramatic reduction in cardiomyocyte numbers. This is in line with gene knockout studies showing reduced cellularity in tissues deleted for survivin, regardless of whether the cells concomitantly survive or die. With apoptosis being unaffected, the reduction in cardiomyocyte numbers in αMHC-survivin−/− mice could thus be explained as being a consequence of cell division defects that impair cardiomyocyte proliferation, which takes place up to several weeks after birth.

Why downregulation of survivin leads to perturbations of mitosis in all cell types but to an increase in apoptosis in only certain ones remains a tantalizing question. This disparity is evident from all tissue-specific survivin knockouts, including ours; whereas deletion of survivin in the brain or endothelium is lethal as a result of massive apoptosis of the respective cell types, its deletion in T cells impairs homeostatic expansion but does not induce apoptosis. In our study, deletion of survivin was not sufficient to drive cardiomyocytes into apoptosis, but its overexpression potently protected them, which is in line with its function as an apoptosis inhibitor. The induction of survivin that we have seen in the failing human heart may serve the same purpose: to protect the cardiomyocyte against apoptosis. Remarkably, survivin is downregulated again after hemodynamic relief by LVAD, implying its potential involvement in the cardiac reverse remodeling process. The increase in [3H]thymidine incorporation and cells within G2/M after survivin overexpression in cardiomyocytes in vitro is remarkable in that it suggests that enforced expression of the gene suffices to induce both DNA synthesis and cell cycle progression. Thus, survivin may have a potentially regenerative function in vivo, a notion that remains to be addressed by the overexpression of survivin in the heart in vivo. Interestingly, in the failing human heart, only individual cardiomyocytes showed strong induction of survivin, whereas the majority did not. Have these cardiomyocytes induced the gene as a protective measure against apoptosis, or are they possibly synthesizing DNA? There is a large body of literature on the question of whether the adult cardiomyocyte has the ability to proliferate. In contrast, the existence of pronounced cardiomyocyte polyploidization in the failing human heart has been known since the 1960s, suggesting the existence of active DNA synthesis. We also have seen polyploidization in the failing...
human heart but, even more remarkably, its dramatic disappearance after LVAD support in the same heart (data not shown). Whether this reduction in DNA content is the result of successful completion of cell division or that of DNA degradation during hemodynamic relief is unknown. However, the mean cardiomyocyte DNA content correlated significantly with survivin immunopositivity in all examined hearts. Thus, survivin may serve as a marker of DNA synthesis, polyploidization, or possibly even cell cycle traverse in the failing human heart.

Loss of cardiomyocytes through cell death regardless of the underlying cause has been implied in the pathogenesis of cardiomyopathies.30 Elegant proof has been provided in transgenic mice expressing low levels of active caspase-8 and Mst-1 in the heart.31,32 In both models, a gradual loss of cardiomyocytes resulting from apoptosis has been implied to be the cause of heart failure and death. Although the apoptotic rate was 0.023% and 0.3%, respectively, neither of the studies had determined the actual numerical extent of cardiomyocyte reduction. Our study may close the gap by providing the actual number of total cardiomyocytes required to maintain normal lifelong cardiac function. We have defined this number as \( \approx 5 \times 10^6 \) cardiomyocytes per heart because below this threshold the individual cardiomyocytes were hypertrophied, reflecting an adaptive response to increased workload. Cellular hypertrophy exponentially increased with declining cardiomyocyte numbers, resulting in up to 7-fold larger cells. The extremely efficient compensation of cell number decline by an increase in cell size may explain why survivin-deficient hearts exhibiting cardiomyocyte numbers below the threshold did not succumb immediately to heart failure but were able to maintain a satisfactory function for months.

Organ size is confined within boundaries considered normal for each species, and it is determined by the number of cells per organ rather than their size.33 The signaling pathways determining cell number during organogenesis are highly conserved and remarkably few.33,34 Among species, the heart weights differ by >1000-fold (\( \approx 0.16 \) g in mice and \( \approx 210 \) g in humans), and although cardiomyocyte size is rather similar (between \( \approx 13\mu m \) diameter in mice and 16-\( \mu m \) diameter in humans), the heart of a mouse contains \( >300 \) fold fewer cardiomyocytes than that of a human (\( 8 \times 10^6 \) versus \( 2600 \times 10^6 \)).35 How utterly important the number of cells in an organ is for its function has been elegantly established for the nervous system, where the number of neurons has been shown to crucially influence brain size, complexity, and possibly enlargement during evolution.36 A similarly crucial role has been proposed for the number of nephrons in the pathogenesis of hypertension; patients with essential hypertension appear to have 50% fewer nephrons than healthy individuals.16 We propose that, correspondingly, the number of “cardiomyocyte working units” per heart may have an impact on the onset, extent, and progression of cardiac diseases by codetermining cardiac function. Because the cardiomyocyte number of each individual is determined during ontogenesis,37 all genetic or environmental factors that influence cardiac development also may influence cardiac cellularity. Such environmental factors may be, for example, alterations in intrauterine nutrition that have been implied in the enhanced susceptibility to cardiovascular diseases in later life.38 Although such “perinatal programming” has been suggested to affect nephron number and thus hypertension,39 from our work, we would suggest that it also affects cardiomyocyte number and thus cardiac function.

Conclusion
Survivin plays a crucial role in controlling cardiomyocyte number during embryonic development and adult life through its profound impact on cardiomyocyte replication and may thus emerge as a new target for myocardial regeneration.

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Disclosures
None.

References
Cardiomyocyte death leading to loss of contractile units has been implicated in the pathogenesis of all forms of heart failure. Although the exact stimuli, mechanisms, and rate of apoptosis in the adult human heart are unknown, a dynamic balance exists between cardiomyocyte loss and replacement during life and disease. We found that the small antiapoptotic molecule survivin controls both cell death and cell division during cardiac development; its deletion in a cardiomyocyte-specific fashion in mice led to progressive heart failure resulting from a profound reduction in total cardiomyocyte numbers per heart. By systematically counting total cardiomyocytes, we were able to determine the minimal cardiomyocyte number sufficient for normal lifelong cardiac function. Survivin overexpression in cultured cardiomyocytes inhibited apoptosis, induced DNA synthesis, and promoted cell cycle progression. In the failing human heart, survivin was potently induced and decreased again after hemodynamic relief through a mechanical left ventricular assist device. We observed that the long-known existence of polyplody of cardiomyocytes in the failing human heart was remarkably decreased after hemodynamic support and correlated with the level of survivin expression at any time. Thus, survivin may be a marker of myocyte DNA synthesis, polyploidy, or possibly even cell cycle traverse in the failing human heart, making it a bona fide target for myocardial regeneration therapies. We suggest that the individual number of “cardiomyocyte working units” that are under the control of survivin may be an independent factor in the susceptibility to cardiac diseases independently of their cause.
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In the article, “Survivin Determines Cardiac Function by Controlling Total Cardiomyocyte Number” by Levkau et al that appeared in the March 25, 2008, issue (Circulation. 2008;117:1583–1593), Dr Fischer’s middle initial was omitted in the byline. Jens Fischer, PhD, should have been listed as Jens W. Fischer, PhD. The current online version of the article has been corrected.

The authors regret this error.

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