Temporaly Controlled Onset of Dilated Cardiomyopathy Through Disruption of the SRF Gene in Adult Heart

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Background—Serum response factor (SRF) is a cardiac transcription factor involved in cell growth and differentiation. We have shown, using the Cre/loxP system, that cardiac-specific disruption of SRF gene in the embryonic heart results in lethal cardiac defects. The role of SRF in adult heart is unknown.

Methods and Results—We disrupted SRF in the adult heart using a heart-specific tamoxifen-inducible Cre recombinase. This disruption led to impaired left ventricular function with reduced contractility, subsequently progressing to dilated cardiomyopathy, as demonstrated by serial echocardiography, including tissue Doppler imaging. The cytoarchitecture of cardiomyocytes was altered in the intercalated disks. All mutant mice died from heart failure 10 weeks after treatment. These functional and structural defects were preceded by early alterations in the cardiac gene expression program: major decreases in mRNA levels for cardiac α-actin, muscle creatine kinase, and calcium-handling genes.

Conclusions—SRF is crucial for adult cardiac function and integrity. We suggest that the rapid progression to heart failure in SRF mutant mice results primarily from decreased expression of proteins involved in force generation and transmission, low levels of polymerized actin, and changes in cytoarchitecture, without hypertrophic compensation. These cardiac-specific SRF-deficient mice have the morphological and clinical features of acquired dilated cardiomyopathy in humans and may therefore be used as an inducible model of this disorder. (Circulation. 2005;112:2930-2939.)

Key Words: cardiomyopathy ■ contractility ■ echocardiography ■ genes ■ heart failure ■ molecular biology ■ myocardium

Diverse forms of hemodynamic stress, such as myocardial infarction, hypertension, and aortic stenosis, result in cardiac compensatory remodeling that can ultimately accelerate functional deterioration and the onset of heart failure (HF). Some stress signals lead to cardiac hypertrophy, which may progress to ventricular dilation, whereas others cause dilation and failure with no intermediate hypertrophic stage.1-5 Generally, mutations affecting proteins of the contractile apparatus give rise to hypertrophic cardiomyopathies, whereas mutations affecting the cytoskeleton give rise to dilated cardiomyopathy (DCM).6-10 Little is known about the signaling pathways that trigger cardiac remodeling. Transcription factors are the downstream effectors of these signaling pathways. Serum response factor (SRF), an MADS-box transcription factor, is involved in the control of extracellular stimulus-regulated genes such as c-fos and of a variety of muscle-specific genes.11,12 In cardiac cells, SRF has been shown to associate with several cardiac transcription factors, including Nkx2.5, TEF-1, and GATA4, in the activation of various cardiac genes.13,14

SRF could be involved in the development of some human cardiomyopathies. An abnormal truncated form of SRF has been found in human failing hearts,15 and the cleavage of SRF by caspase 3 may promote HF.16 In mice, classic disruption of the SRF gene leads to defective mesoderm formation and death early in embryogenesis.17 We previously used a Cre/loxP strategy to demonstrate that SRF is crucial for cardiac differentiation and maturation during embryogenesis.18 Similar results were recently obtained by Miano et al.19 In this study we investigated the role of SRF in adult cardiac function by triggering SRF

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loss through the use of \(\alpha\)-MHC-MerCreMer mice, which produce a tamoxifen-inducible Cre recombinase.\textsuperscript{20} SRF inactivation in the hearts of adult mice leads to progressive impairment of cardiac function, with reduced contractility, progressing to DCM, HF, and death within 10 weeks. We observed changes in the cardiac gene expression program during progression to HF, demonstrating that SRF is crucial for adult cardiac function and integrity.

**Methods**

**Transgenic Mice**

Mice homozygous for SRF-floxed alleles\textsuperscript{18} (abbreviated Sf/Sf) and transgenic \(\alpha\)-MHC-MerCreMer mice\textsuperscript{20} were backcrossed with C57BL/6J mice for at least 4 generations and crossed to generate double-transgenic (\(\alpha\)-MHC-MerCreMer:Sf/Sf) mice. Groups of 2-month-old \(\alpha\)-MHC-MerCreMer:Sf/Sf and control (Sf/Sf) mice were given daily intraperitoneal tamoxifen (20 \(\mu\)g/g per day; Sigma) injections on 4 consecutive days, with the day of the first injection counted as day 1. This study conformed to institutional guidelines for the use of animals in research.

**Echocardiography**

Echocardiography was performed as previously described\textsuperscript{21–23} with a Toshiba Powervision 6000, SSA 370A device equipped with an 8- to 14-MHz linear transducer under isoflurane anesthesia (0.75% to 1.0% in oxygen) with spontaneous ventilation. The body temperature was maintained with a heating pad. Data were transferred online to a computer for offline analysis (Ultrasound Image Workstation-300A, Toshiba). The left ventricle (LV) was imaged in parasternal long-axis view to obtain measurements of the left atrium (LA) and LV (LV end-diastolic diameter, shortening fraction, ejection fraction) in time-motion mode. Pulsed-wave Doppler tissue images were obtained from the posterior wall for the measurement of maximal...
systolic wall velocities. The apical view was used for pulsed-wave Doppler measurements of LV mitral inflow (E wave) and LV aortic outflow (ejection time), and time-motion color Doppler mode (E wave propagation velocity [EpV]) and for tissue Doppler measurement of mitral annulus velocities (systolic wave [Sa] and diastolic wave [Ea]). E/Ea was computed as an estimate of LV filling pressure.24

Histological Analysis and Immunohistochemistry

Histological analysis and immunohistochemistry were performed as previously described.18 Paraffin-embedded sections were incubated with anti-SRF antibody (1:100, Santa Cruz) followed by biotin-streptavidin–horseradish peroxidase (HRP) kit (Vector Laboratory). Frozen sections were incubated with the following primary antibodies: anti-vinculin (1:400, hVIN-1, Sigma), anti-actinin (1:800, EA-53, Sigma), anti-desmin (1:50, D33, Dako), anti–pan-cadherin (1:400, CH-19, Sigma) and anti–connexin 43 antibody (1:400, Chemicon), anti–β-actin (1:500, AC-15), and anti–sarcomeric actin (1:80, d-SR-1) (Sigma). HRP-conjugated secondary anti-rabbit (1:3000) or anti-mouse (1:2000) antibody (Dako) was used to detect the proteins by ECL. Western blot analysis of sarcomeric actin was also performed on soluble and insoluble protein extracts with the use of buffers of different ionic strengths. Hearts were homogenized in a buffer (150 mmol/L NaCl, 5 mmol/L EDTA, 1% Tween-20, 50 mmol/L Tris, pH 7.4, protease inhibitor cocktail [Roche]), incubated for 30 minutes on ice, sonicated, and then centrifuged for 1 hour at 25 000g. Pellets were suspended in high-ionic strength buffer (1.5 mol/L NaCl) to extract insoluble fractions.

TABLE 2. Echocardiographic Data for a Separate Group of Mice 21 Days After Tamoxifen Injection

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>Mutant (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>24.3 ± 1.8</td>
<td>27.9 ± 1.29</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>499 ± 9.9</td>
<td>527 ± 17.9</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>4.27 ± 0.11</td>
<td>4.26 ± 0.2</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>0.62 ± 0.05</td>
<td>0.63 ± 0.01</td>
</tr>
<tr>
<td>EF, %</td>
<td>79 ± 2</td>
<td>67 ± 1.1*</td>
</tr>
<tr>
<td>VcFc, circumferences/s</td>
<td>2.88 ± 0.17</td>
<td>2.14 ± 0.02*</td>
</tr>
<tr>
<td>Sa, cm/s</td>
<td>2.77 ± 0.18</td>
<td>2.51 ± 0.19</td>
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<tr>
<td>Spw, cm/s</td>
<td>2.60 ± 0.13</td>
<td>2.62 ± 0.26</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>17.2 ± 0.72</td>
<td>19.2 ± 1.21</td>
</tr>
<tr>
<td>Epv, cm/s</td>
<td>58.5 ± 10.7</td>
<td>58.2 ± 6.92</td>
</tr>
<tr>
<td>E/Ea</td>
<td>20.4 ± 0.87</td>
<td>19.6 ± 1.33</td>
</tr>
</tbody>
</table>

LVEDD indicates LV end-diastolic diameter; EF, ejection fraction; VcFc, mean shortening velocity of circumferential fibers corrected for heart rate; Sa, systolic mitral annulus maximal velocity; Spw, systolic maximal velocity of the posterior wall; IVRT, isovolumic relaxation time; EpV, E wave propagation velocity; and E/Ea, blood to mitral annulus diastolic velocity ratio.

*P<0.05.

Efficient Tamoxifen-Induced Loss of Cardiac SRF α-MHC-MerCreMer:S/S' double-transgenic mice were healthy, with no sign of cardiac dysfunction (as assessed by echocardiography). Two-month-old S/S' (controls) and α-MHC-MerCreMer:S/S' mice were given daily injection of tamoxifen for 4 consecutive days to excise exon 2 from the floxed SRF alleles (Figure 1a). RT-PCR on RNA from the hearts of mutant mice 5 and 60 days after tamoxifen treatment showed a marked decrease in SRF mRNA levels as early as day 5 (Figure 1b). Levels of SRF protein also decreased dramatically in the hearts of mutant mice (Figure 1c). More than 90% of cardiomyocyte nuclei were not immunostained for SRF than 90% of cardiomyocyte nuclei were not immunostained.

TABLE 1. Heart Rate, Body Weight, and LV Posterior Wall Thickness of Animals Described in Figure 2

<table>
<thead>
<tr>
<th></th>
<th>0 Day After Tamoxifen Injection</th>
<th>30 Days After Tamoxifen Injection</th>
<th>60 Days After Tamoxifen Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>23.7 ± 2.05</td>
<td>22.6 ± 1.26</td>
<td>24.97 ± 1.22</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>490 ± 15.8</td>
<td>497 ± 12.4</td>
<td>560 ± 11.9</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>0.54 ± 0.03</td>
<td>0.56 ± 0.06</td>
<td>0.63 ± 0.04</td>
</tr>
</tbody>
</table>

LVPW indicates LV posterior wall thickness.

Results

Statistical Analysis

We performed ANOVA and Fisher post hoc tests for repeated measures and used unpaired Student t tests for comparisons with control mice at a given time point. The data shown are mean±SEM. A value of P<0.05 was considered significant.

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for SRF 1 month after tamoxifen treatment (Figure 1d), whereas SRF was not inactivated in the vascular smooth muscle cells, demonstrating the specificity of α-MHC-MerCreMer for cardiomyocytes.

**SRF Mutants Develop DCM**

We assessed cardiac function in α-MHC-MerCreMer:S'/S' (mutant) mice and control littermates by serial echocardiography just before (0 day) and after (21, 30, and 60 days) tamoxifen treatment. Heart rate was kept as close as possible to physiological levels for echocardiography (Table 1). There was no difference between control and mutant mice before treatment (Figure 2). On day 21, a significant decrease in LV contractility was evidenced in mutant mice (Table 2, ejection fraction and mean shortening velocity of circumferential fibers corrected for heart rate), with no detectable change in diastolic LV parameters (isovolumic relaxation time, EpV, E/Ea). One month after treatment, mutant mice had mild LV enlargement (LV end-diastolic diameter) without significant LA remodeling and moderately increased LV mass index (Figure 2a, 2b). Echographic parameters of contractility (ejection fraction, mean shortening velocity of circumferential fibers corrected for heart rate, systolic mitral annulus maximal velocity, and systolic maximal velocity of the posterior wall) and relaxation (isovolumic relaxation time, EpV) were moderately altered (Figure 2c, 2d). However, no sign of HF was observed as LV filling pressure did not differ from that in controls (E/Ea).

In contrast, 2 months after tamoxifen treatment, functional LV assessment showed a massive decrease in LV contractility and LV relaxation (Figure 2c, 2d). E/Ea ratio had clearly increased at this time point, indicating overt HF.

The hearts of mutant mice had enlarged considerably 2 months after injection (Figure 3a to 3d). Total heart weight to body weight ratio was 7.2±1.3 mg/g (n=8) in SRF mutant mice and 5.54±0.39 mg/g (n=10) in the control mice, whereas mutant and control mice had presented similar heart weight to body weight ratios at 1 month. However, echocardiography and histology revealed that this increase in heart weight to body weight ratio was not associated with thickening of the free wall (Table 1, Figure 3). Instead, SRF mutant hearts displayed the anatomic features of eccentric hypertrophy, with dilation of the ventricular chambers. The organ weight to body weight ratios for other organs (kidney, liver, lungs) were unaffected. There were frequently larger spaces between cardiomyocytes in mutant than in control hearts (Figure 3e, 3f), indicating alterations in interstitial and mild fibrosis in subendocardial regions in mutant mice (Figure 3g, 3h). Mutant mice appeared sick and became inactive a few days before death. All SRF mutant mice (n=10) died 8 to 10 weeks after tamoxifen injection, indicating rapid progression toward overt HF. Thus, functional and histological assessment of the mutant mice recapitulated the features of DCM with an early defect in contractility (21 days) and progression toward overt HF (60 days) and no evidence of intermediate concentric hypertrophy.

![Figure 3. Morphological and histological analysis. a and b, Hearts of control and mutant mice 60 days after tamoxifen injection. c and d, Hematoxylin-eosin staining of paraffin-embedded heart sections. Left (lv) and right ventricles (rv) of the mutant heart have dilated lumens compared with control sections (bars=2 mm). e and f, Higher magnification of fresh-frozen sections shows the presence of frequent intercellular gaps (*) in mutant hearts (bar=10 μm). g and h, Cirrus red staining of cardiac tissue shows the presence of subendocardial fibrotic regions (arrows) in mutant mice (bar=156 μm).](http://circ.ahajournals.org/)

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Dysregulated Gene Expression in SRF Mutant Mice

We assessed the consequences of SRF loss for the cardiac gene expression by Northern blot and quantitative RT-PCR 0, 5, 30, and 60 days after tamoxifen treatment (Figure 4). We confirmed the efficient loss of SRF gene expression. We observed a rapid, major decrease in expression of cardiac α-actin (Figure 4a, 4b). Skeletal α-actin and α-MHC mRNA (Figure 4b) levels also decreased, but to a lesser extent. Levels of the mRNAs encoding β-MHC and β-actin decreased slightly at an early stage (day 5) but returned to normal later. We also quantified several mRNAs encoding cytoskeleton and actin-associated proteins. Desmin, vinculin, and zyxin mRNAs were clearly induced on day 60, whereas only a transient decrease in mRNA levels was observed for talin, coflin-2, and gelsolin on day 5. No effect on thymosin-β4 mRNA levels was observed.

Genes involved in energy flux and calcium handling were also affected. MCK, a known target of SRF, was progressively downregulated after SRF inactivation. In contrast, the responses to SRF loss of sarcomeric mitochondrial CK (ScCKmt) and B-CK were complex, with an initial decrease in expression on day 5, a return to normal on day 30, and a second decrease on day 60. The Serca2 gene, which harbors a functional CArG box in its upstream promoter, also displayed a 2-phase response. Finally, the amount of RyR2 mRNA increased at the end stage, whereas the amount of phospholamban mRNA decreased slightly.

We previously reported that the loss of SRF during cardiac development results in a decreased production of several cardiac transcription factors. The loss of SRF did not result in a significant change in expression of the Nkx2.5 gene in adult heart, whereas TEF1 expression increased continuously from early to late stages (Figure 4a). The amounts of mRNA for MEF2C and GATA4 were increased only on day 60. The amount of myocardin, a cofactor of SRF, first decreased (day 5) and then increased. Expression of genes encoding cytokines involved in cardiomyocyte growth and survival, such as IGF1 and TGFβ1, was transiently decreased on day 5 but upregulated during the end stage of disease (Figure 4b). The stress-induced gene ANF, which has CArG boxes in its promoter, was downregulated at an early stage of SRF loss but strongly expressed on day 60 (Figure 4a).

Altered Cardiac Cytoarchitecture in SRF Mutant Mice

We examined the amount of actin and the organization of sarcomeres and intercalated disks, involved in force generation and transmission, on frozen sections from mutant mice and controls 21, 30, and 60 days after tamoxifen treatment. The amount of polymerized F-actin in the cardiomyocytes was assayed by phalloidin-TRITC staining and quantified in gain- and depth-matched confocal sections of controls and mutant myocardium (Figure 5a to 5d). The density of phalloidin staining 21 days after tamoxifen injection was 20% lower in mutant hearts than in control hearts (Figure 5q). Phalloidin signal intensity continued to decrease with disease progression, falling to 50% control levels. By contrast, phalloidin signal intensity was not affected in the vascular smooth muscle cells of the mutant.

Immunostaining for vinculin, a component of intercalated disks, revealed major differences between mutant and control hearts at late stages (Figure 5e to 5j). The intercalated disks of mutant hearts were dramatically enlarged.
and irregularly shaped compared with the rectangular shape of control intercalated disks at 60 days. This phenotype progressed quantitatively and qualitatively, with 10.5% and 36.4% of intercalated disks showing slight enlargement at 21 and 30 days, respectively. At 60 days, 95% of intercalated disks were highly enlarged. Mutant intercalated disks were much thicker (3.44 ± 0.24 versus 1.15 ± 0.09 μm) than control intercalated disks at 60 days (Figure 5r). Similar results were obtained with desmin (Figure 5o, 5p) and pan-cadherin (data not shown). Connexin-43 followed the expansion of the intercalated disks but maintained its punctuate distribution pattern in the mutant mice (Figure 5k, 5l). Finally, mutant hearts showed an irregular alignment of cardiomyocytes, with frequent gaps between cells (Figure 5h, 5j). Immunostaining for sarcomeric α-actinin and desmin (Figure 5m to 5p) showed that the Z disks were misaligned and the myofibrils split in the cardiomyocytes of the mutant SRF mice at 60 days. The cross-sectional area of the cardiomyocyte, as measured on phallolidin-stained sections, was similar in mutant and control mice from day 0 to day 60 (Figure 5s), supporting the hypothesis that compensatory concentric hypertrophy does not occur in the SRF mutant. Electron microscopy of mutant cardiac tissue at 60 days showed a lower density of myofibrils and defect in demarcation of Z lines and M lines (Figure 6a, 6b). The structure of intercalated disks was altered in mutant mice, with extensive interdigitation and the presence of lacunae, a widened space at the site of myofibril attachment to the intercalated disks. The myofibrils at the intercalated disks were often stretched and fragmented (Figure 6c, 6d) in mutant hearts.

**Changes in Protein Profile Associated With DCM in SRF Mutant Mice**

Several cytoarchitectural proteins were analyzed at various times after SRF inactivation. At 8 days, the profiles of these

![Figure 5. Confocal microscopy of cardiac sections.](image)
proteins were similar in SRF mutant and control mice. However, the levels of connexin-43, vinculin, and β-actin had increased when overt HF occurred (Figure 7a). As expected, total sarcomeric α-actin levels were found to have decreased by this stage. More sarcomeric α-actin was found in the soluble fraction extracted from SRF mutant hearts than in that from controls at 60 days. However, less sarcomeric α-actin was found in pellets from SRF mutant hearts than in pellets from control hearts (Figure 7b). The ratio of insoluble (polymerized F-actin) to soluble (unpolymerized) actin was therefore lower in the SRF mutant hearts. This finding is consistent with the weak phalloidin staining observed in sections of heart from mutant mice at a late stage of DCM (Figure 5d).

Discussion
Altered Cardiac Gene Expression Program in the Absence of SRF
In this study we used an inducible Cre-mediated SRF disruption strategy to assess for the first time the role of SRF in adult heart. The loss of SRF led to HF with DCM, characterized by a progressive loss in contractility and LV dilation. Our study revealed an unexpected complexity in the transcriptional response to SRF inactivation. Indeed, some cardiac genes with established SRF-binding sites—cardiac and skeletal α-actin and MCK—were rapidly and progressively downregulated in the absence of SRF. This downregulation paralleled the functional impairment and demonstrated the strict requirement of SRF for the transcription of these genes in adult cardiomyocytes. In contrast, the level of transcription of α-MHC and β-MHC, both of which have been reported to be targets of SRF in vitro, was only slightly lower in mutant than in control hearts. In contrast to reports in regard to SRF-ES cells, no change in mRNA levels for vinculin and zyxin was observed in mutant hearts during the first 30 days, with a large increase at the time of overt HF. This suggests that the expression of these genes in normal adult cardiomyocytes is not strictly dependent on SRF. The temporal pattern of expression of other SRF target genes, such as ANF and Serca2, is more complex, with early downregulation followed by a transient return to normal levels of expression at 1 month and final downregulation at end-stage HF for Serca2 and strong late induction for ANF. Such complex patterns of regulation may be accounted for by the effect of other transcription factors involved in the adaptation of the heart to progressive functional changes. Indeed, the inactivation of SRF results in the upregulation of TEF1, MEF2, and GATA4, which regulate α-MHC and Serca2 and could partly compensate for the lack of SRF. TEF1 expression is increased from the early stage to late stage, suggesting that SRF directly or indirectly represses TEF1 activation in adult cardiomyocytes.

Downregulation of Cardiac α-actin Synthesis and Treadmilling in SRF Mutant Mice
Actin filaments form highly ordered, relatively stable structures that, together with myosin, form the basic contractile unit of muscle cells. Actin also attaches to Z disks and
intercalated disks and is therefore also involved in force transmission from cell to cell. Indeed, some inheritable forms of human DCM are linked to mutations specifically affecting the domains of actin that attach to Z bands and intercalated disks. Cardiac actin is the main component of the thin filament of the sarcomere. Inactivation of SRF in the heart decreases cardiac actin mRNA levels by 75%, which may disturb actin turnover by preventing the synthesis of new actin, in turn decreasing force generation and transmission. Total sarcomeric actin protein levels are lower than normal in SRF mutant hearts. Our results suggest that actin dynamics are also affected by the absence of SRF because the proportion of unpolymerized actin increased in late stages of DCM. This finding is consistent with studies on embryonic stem cells lacking SRF, which have shown a shift in actin treadmilling equilibrium from F-actin toward G-actin and a decrease in overall actin concentration. The reason for the decrease in polymerized actin levels is unclear. Modifications to actin-associated proteins and the RhoA signaling pathway may affect actin dynamics. Hence, changes in cardiac actin synthesis and dynamics may be primary alterations in the DCM of SRF mutant mice.

Altered ID Architecture and Cardiac Dilation in SRF Mutant Mice

Intercalated disks seem to be modified progressively after SRF excision, with these modifications occurring after the downregulation of polymerized actin production. Aberrant intercellular spaces are also frequent in SRF mutant mice, suggesting diminished interaction between cardiomyocytes, another hallmark of DCM. Hereditary forms of DCM have been reported to be associated with mutations in the cytoskeleton proteins involved in anchoring or laterally integrating myofibrils, such as dystrophin, dystrophin-associated proteins, and desmin. Work with animal models, such as MLP knockout mice and cadherin-misexpressing mice, has suggested that defects in mechanical stress sensor pathways due to changes in intercalated disk organization may play a crucial role in the development of DCM. Indeed, the induced deletion of N-cadherin in the adult heart was recently shown to result in DCM. The intercalated disks are the sites of actin anchorage and contribute to force transmission by linking the contractile apparatus of adjacent cells. The lower polymerized actin content of the hearts of SRF mutants probably leads to secondary alterations in intercalated disk architecture and function.

Altered Energy Flux and Calcium Handling in SRF Mutant Mice

Another remarkable feature of SRF mutant mice is their low MCK mRNA levels. Creatine kinase (CK)–deficient hearts are more susceptible to ischemia/reperfusion injury and impaired calcium homeostasis than normal hearts. CK activity is greatly depressed in severe HF, with this decrease in activity mostly affecting MCK and scCKmt. The MCK mRNA level of SRF mutant mice progressively decreased and was very low (10%) at late stages of the disease, whereas ScCKmt mRNA level decreased by 60%. SRF mutant mice also have lower than normal mRNA levels for Serca2 and higher than normal RyR2 content, potentially causing additional calcium-handling defects and contributing to the development of HF.

Loss of SRF in Adult Cardiomyocytes and HF

We propose a 2-hit model for the onset of DCM and rapid progression toward HF in the absence of SRF. The first main hit is a drastic decrease in actin synthesis and profound alterations in the expression of other genes, leading to a decrease in contractile function as soon as 3 weeks after SRF depletion. The second hit would be a tentative compensatory response triggered by the decrease in cardiac contractility, as indicated by the return to normal of mRNA levels for several genes such as Serca2, RyR2, myocardin, and ScCKmt and the increase in the production of...
of factors such as IGF1 and TGFβ observed 1 month after tamoxifen injection. However, this response does not result in hypertrophic compensation in the absence of SRF. Consistent with the hypothesis that SRF is required for hypertrophic growth, the overexpression of SRF gene in mouse heart leads to concentric hypertrophic cardiomyopathy,38 whereas the overproduction of a dominant negative mutant form of SRF leads to severe dilation and death early in postnatal life, when physiological growth is required to increase cardiac mass.39 These findings are consistent with SRF being an important mediator of both physiological growth and pathological hypertrophy. The biomechanical stress caused by the decrease in cardiac performance further increases the strain on cardiomyocytes, as shown by the major changes in myofibrils and intercalated disks at late stages, promoting terminal HF.

Our scheme of SRF inactivation in the adult heart circumvents the adaptive changes that may occur in conventional transgenic models. Our cardiac-specific SRF mutant mice thus provide a new inducible model suitable for studying the chain of molecular events leading to acquired DCM that is a multifactorial and polygenic disease.

Acknowledgments

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References

Clinical Perspective

Currently there is great interest in identifying the signaling pathways and their downstream transcriptional circuitry that govern cardiac gene expression patterns involved in myocardial remodeling and progression of left ventricular dysfunction in human heart failure, allowing drug discovery in well-defined animal models. The relevance of the transcription factor serum response factor (SRF) in human pathology has recently been suggested by the demonstration of impaired SRF activity in the failing heart, apparently through caspase-mediated cleavage of SRF leading to the production of a naturally occurring, dominant negative form. In the present report we demonstrate that temporally controlled inactivation of SRF in cardiac tissue leads to a progressively reduced contractility and dilated cardiomyopathy, with mutant mice succumbing from heart failure at 10 weeks after gene deletion. Mutant mice displayed major decreases in mRNA levels for cardiac α-actin, muscle creatine kinase, and calcium-handling genes. Mutant mice displayed an extensive interdigitation of intercalated disks, which is a characteristic feature of dilated cardiomyopathies in humans. This work demonstrates that disruption of SRF in the adult heart is sufficient to induce heart failure and sheds light on the mechanisms by which SRF controls cardiac function and integrity. This murine model mimics human dilated cardiomyopathy and could be a physiologically relevant heart failure model useful for testing pharmacological intervention strategies.
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