Follistatin-Like 1 Is an Akt-Regulated Cardioprotective Factor That Is Secreted by the Heart
Yuichi Oshima, MD, PhD; Noriyuki Ouchi, MD, PhD; Kaori Sato, MD, PhD; Yasuhiro Izumiya, MD, PhD; David R. Pimentel, MD; Kenneth Walsh, PhD

**Background**—The Akt protein kinase is an important mediator of cardiac myocyte growth and survival. To identify factors with novel therapeutic applications in cardiac diseases, we focused on the identification of factors secreted from Akt1-activated cells that have cardioprotective effects through autocrine/paracrine mechanisms.

**Methods and Results**—Using an inducible Akt1 transgenic mouse model, we have found that follistatin-like 1 (Fstl1) protein and transcript expression are increased 4.0- and 2.0-fold, respectively, by Akt activation in the heart (P<0.05). Fstl1 transcript was also upregulated in response to myocardial stresses including transverse aortic constriction, ischemia/reperfusion injury, and myocardial infarction. Adenovirus-mediated overexpression of Fstl1 protected cultured neonatal rat ventricular myocytes from hypoxia/reoxygenation-induced apoptosis (P<0.01), and this protective effect was dependent on the upregulation of both Akt and ERK activities. Conversely, knockdown of Fstl1 in cardiac myocytes decreased basal Akt signaling and increased the frequency of apoptotic death in vitro (P<0.01). The intravenous administration of an adenoviral encoding Fstl1 to mice resulted in a 66.0% reduction in myocardial infarct size after ischemia/reperfusion injury that was accompanied by a 70.9% reduction in apoptosis in the heart (P<0.01).

**Conclusions**—These results indicate that Fstl1 is a cardiac-secreted factor that functions as an antiapoptotic protein. Fstl1 could play a role in myocardial maintenance and repair in response to harmful stimuli. *(Circulation. 2008;117:3099-3108.)*

**Key Words:** apoptosis  ■ myocytes  ■ reperfusion

The serine/threonine protein kinase Akt is a key regulator of myocardial growth that is essential for cardiac adaptation to diverse stress.1 The cardioprotective and antiapoptotic properties of Akt have been documented in cultured cardiac myocytes2 and in myocardial ischemia/reperfusion and heart failure models.3,4 Furthermore, phosphatidylinositol 3-kinase (PI3K)-Akt signaling plays important roles in cardiac tissue growth.5 Work from a number of laboratories has shown that cardiac-specific overexpression of modified forms of Akt1 or Akt3 will have different effects on heart phenotype ranging from mild hypertrophy and increased cardiac function to extensive hypertrophy associated with interstitial fibrosis and contractile dysfunction.5,8 In addition, overexpression of nuclear localization of a nuclear-targeted form of nonactivated Akt1 increases contractile function and protects myocytes from apoptosis.9,10

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A growing body of evidence shows that the heart secretes factors to maintain its performance. Examples include atrial natriuretic peptide, brain natriuretic peptide, cardiotrophin-1, and adrenomedullin.11–15 More recently, Frost and Engelhardt16 employed a yeast secretion trap screen to identify a novel antihypertrophic protein that is secreted by the heart. We have reported that physiological cardiac hypertrophy in Akt transgenic mice is dependent on vascular endothelial growth factor secretion and the coupling of coronary angiogenesis with myocyte growth.8 Furthermore, it has been reported that the injection of Akt-transduced mesenchymal stem cells into infarcted rat hearts leads to an improvement in cardiac function that results from the release of paracrine factors from these cells rather than through their limited ability to fuse with or differentiate into myocytes.17 These data indicate that the autocrine/paracrine effects of factors secreted from Akt-activated cells could play significant roles in cardiac growth and maintenance.

Previously, we constructed a cardiac-specific inducible Akt1 transgenic mouse system that produces a physiological form of hypertrophy after short-term induction and a pathological form of hypertrophy after long-term induction.8 To
elucidate the mechanisms by which Akt signaling regulates cardiac phenotype, we performed an Affymetrix microarray analysis on transgenic mouse hearts at different time points after Akt induction to identify differentially regulated transcripts. One of these transcripts, follistatin-like 1 (Fstl1), is a member of the follistatin family of factors that bind to transforming growth factor-β (TGF-β) superfamily proteins. Transfection of Fstl1 into cancer cell lines is reported to inhibit proliferation and invasive behavior, but there are no functional studies on this factor in the heart. Here we report that Akt transgene activation upregulates the expression and secretion of Fstl1 in cardiac myocytes. We also show that Fstl1 is upregulated by injury in the heart and that it functions as a cardioprotective molecule in vitro and in vivo.

Methods
An expanded Methods section can be found in the online-only Data Supplement.

Transgenic Mice
The generation of cardiac-specific inducible myrAkt1 transgenic mice was described previously. Briefly, 2 transgenic mouse lines (Tet-myrAkt1 and α-MHC-tTA) were mated to generate double-transgenic mice (DTG), and these mice were maintained with 0.5 mg/mL doxycycline in the drinking water, resulting in the repression of myrAkt1 gene expression. When doxycycline is withdrawn from the drinking water, tTA binds to tetO elements and induces myrAkt1 gene expression in the cardiac myocytes. For these studies, the Akt1 transgene was induced for 2 weeks by withdrawing doxycycline from the drinking water at the age of 12 weeks. Control experiments were performed with MHC-tTA single-transgenic mice that underwent the same doxycycline treatment protocol as DTG mice.

Microarray Analysis
Microarray analysis were performed by Affymetrix GeneChip Mouse Expression Set 430 microarrays and normalized as described in a previous report. Gene expression levels were compared before myr-Akt1 induction and 2 weeks after myr-Akt1 induction. Among transcripts that are upregulated by Akt activation, we selected transcripts that have full-length open reading frame cDNAs available in the National Center for Biotechnology Information Web site (http://www.ncbi.nlm.nih.gov/). Amino acid sequences were then examined for signal sequences with the use of SignalIP software. Transcripts with signal sequences were further analyzed with Sosui signal beta version software (http://bp.nuap.nagoya-u.ac.jp/sosui/) to predict transcripts with transmembrane domains.

Cloning and In Vitro Transfection Assay of Mouse Fstl1
Full-length Fstl1 cDNA was obtained by polymerase chain reaction (PCR) of RNA isolated from cardiac myocytes and subcloned into pcDNA3.1/V5-His that express mouse Fstl1 as a fusion to the V5 epitope at the C-terminus. To test the secretion of the gene product, the plasmid vector pcDNA3.1/V5-His expressing Fstl1 was transfected into HEK293 cells with the use of Lipofectamine2000 (Invitrogen, Carlsbad, Calif.). After cells were incubated with serum-free media for 24 hours, the cell lysate and media were collected. Cells were mock-transfected (no plasmid) as a negative control. The collected media was concentrated ~10-fold with the use of Microcon (Millipore). Cell lysates and media were separated by SDS-PAGE, and Fstl1 fused with V5 was detected by Western blot analysis with anti-V5 antibody (Invitrogen).

Construction of Adenoviral Vector Expressing Mouse Fstl1
Full-length mouse Fstl1 cDNA was subcloned into an adenovirus shuttle vector. After linearization, shuttle vector was cotransformed into Escherichia coli with the adenovirus backbone plasmid pAdEasy-1. The resultant recombinant adenoviral DNA with Fstl1 cDNA was transfected into HEK 293 cells to produce the recombinant adenoviral vector. For some experiments, an adenoviral vector expressing β-galactosidase (Ad-βgal) was used as a control. Adeno-viral vectors were purified by CsCl ultracentrifugation.

RNA Interference in Ventricular Myocytes
The rat Fstl1 small interfering RNA (siRNA) Smart Pool was purchased from Dharmacon Inc, and the second siRNA targeting Fstl1 and unrelated siRNA were from Qiagen. The sequences of siRNAs used in this study were as follows: Fstl1 (Dharmacon): mixture of 4 siRNAs, 5’-UGCAAAUAUCUACGGACUUUU-3’, 5’-CAGAUGGGACUGAGACCGAUU-3’, 5’-CCGUCAAACAUCGGCUAAUU-3’, and 5’-UGCUAAGCCGCGAGCGCAU-3’; and Fstl1-b (Qiagen): 5’-r(GCAUCUUGAGAUUUACA)dTdT-3’. Neonatal rat ventricular myocytes (NRVMs) were transfected with siRNA by Lipofectamine 2000 according to the manufacturer’s protocol. Forty-eight hours after transfection, the protein or mRNA was extracted for Western blot analysis or quantitative real-time polymerase chain reaction (QRT-PCR) or cells were exposed to hypoxia/reoxygenation.

Statistical Analysis
Data are presented as mean±SEM. Group differences were analyzed by 2-tailed Student t test or ANOVA. To compare multiple groups, the Mann–Whitney U test with Bonferroni correction was used. A value of P<0.05 was considered statistically significant.

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

Results
Fstl1 Upregulation by Akt Activation in the Heart
Microarray gene expression analysis was compared between control mouse hearts and in hearts 2 weeks after myocardial induction of myristoylated Akt1 by withdrawing doxycycline from the drinking water of DTG mice. Transcripts upregulated by 2-week Akt activation with full-length open reading frame cDNAs were selected. Amino acid sequences were then examined for signal sequences with the use of Signal IP software. Transcripts with signal sequence were further analyzed with Sosui signal beta version software to exclude transcripts predicted to encode proteins with transmembrane domains. Akt-regulated transcripts were identified that contained a predicted signal sequence but lacked a transmembrane domain. Of this subset of proteins, Fstl1 was selected for further analysis because it was upregulated 2.7-fold. To confirm the changes in Fstl1 mRNA level, QRT-PCR was performed with specific primer sets for Fstl1 and GAPDH (Table). Fstl1 was upregulated 2.0-fold by Akt activation in the heart (P<0.05). In contrast, transcripts encoding other proteins containing the follistatin domain, including follistatin, follistatin-like 3 (Fstl3), and secreted acidic cysteine-rich glycoprotein (SPARC), were not regulated by activation of the Akt transgene in hearts analyzed as assessed by microarray and QRT-PCR analyses (data not shown and Table, respectively). Fstl1 transcript and protein expression in mouse hearts were not affected by the presence or absence of doxycycline in the drinking water of control mice (data not shown).
To better understand the regulation of Fstl1 in cardiac cells, transcript levels of this factor were measured by RT-PCR in hearts that were subjected to various injuries. Fstl1 transcript was upregulated ~7-fold at the 7-day time point after transverse aortic constriction that induces pressure overload hypertrophy (Figure 1A). Fstl1 transcript was upregulated ~2-fold in the myocardial area at risk 1 day after ischemia/reperfusion injury (Figure 1B) and ~13-fold in hearts 3 days after permanent left anterior descending coronary artery (LAD) ligation (Figure 1C). Fstl1 protein levels were analyzed by Western immunoblot analysis after myocardial infarction because the changes in transcript level were robust in this model. LAD ligation led to a substantial increase in Fstl1 protein in the heart (Figure 1D). Fstl1 immunoreactive material appeared as a doublet with electrophoretic mobilities indicative of 37- and 46-kDa proteins. Quantification of the 37-kDa band indicated a 10-fold upregulation of the Fstl1 protein (Figure 1E). Fstl1 protein could also be detected in the serum after Western immunoblot with an electrophoretic mobility that corresponded to that of a 46-kDa protein. After myocardial infarction, the 46-kDa form of Fstl1 increased 3-fold in serum (Figure 1F). Thus both Fstl1 protein and transcript are induced by a variety of stresses in the heart.

Figure 2A shows Western immunoblots from 2 control hearts subjected to sham surgery or myocardial infarction because the changes in transcript level were robust in this model. LAD ligation led to a substantial increase in Fstl1 protein in the heart (Figure 1D). Fstl1 immunoreactive material appeared as a doublet with electrophoretic mobilities indicative of 37- and 46-kDa proteins. Quantification of the 37-kDa band indicated a 10-fold upregulation of the Fstl1 protein (Figure 1E). Fstl1 protein could also be detected in the serum after Western immunoblot with an electrophoretic mobility that corresponded to that of a 46-kDa protein. After myocardial infarction, the 46-kDa form of Fstl1 increased 3-fold in serum (Figure 1F). Thus both Fstl1 protein and transcript are induced by a variety of stresses in the heart.

Table. Upregulation of Fstl1 Transcript by Cardiac Myocyte-Specific Overexpression of Akt

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold Increase (Versus Control)</th>
</tr>
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<tbody>
<tr>
<td>Fstl1</td>
<td>2.04 ± 0.10 ^*</td>
</tr>
<tr>
<td>Follistatin</td>
<td>0.36 ± 0.13</td>
</tr>
<tr>
<td>Fstl3</td>
<td>0.68 ± 0.08</td>
</tr>
<tr>
<td>SPARC</td>
<td>0.73 ± 0.07</td>
</tr>
<tr>
<td>Akt1</td>
<td>7.19 ± 0.83 ^*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE, n = 3 for each group. ^*P < 0.05 compared with control.
Fstl1 Is a Secreted Protein
Plasmid and adenovirus vectors expressing Fstl1 were constructed. With the use of cDNA mouse heart, Fstl1 was subcloned into the pcDNA3.1/V5-His expression vector. The vector was transfected into HEK293 cells, and cells were incubated with serum-free media for 24 hours. As shown in Figure 3A, the tagged Fstl1 protein was detected in both the cell pellet lysate and the media, indicating that it is secreted from HEK293 cells. The apparent molecular weight of Fstl1, based on electrophoretic mobility, was greater in the media than in the cell pellet lysate.

An adenoviral expression vector expressing nontagged Fstl1 was also constructed and tested. NRVMs were transduced with Ad-Fstl1 or Ad-βgal, as a control, and Fstl1 protein was detected in both the cell pellet and the media (Figure 3B). In addition, lower-intensity Fstl1 signals, corresponding to endogenous Fstl1 expression, could be detected in the lysate and media of cells transduced with Ad-βgal (Figure 3B). As with the plasmid-encoded Fstl1 produced by HEK293 cells, the protein detected in the NRVM media exhibited reduced electrophoretic mobility, indicating that the secreted protein is posttranslationally modified. The predicted molecular weight of the protein in the NRVM pellet corresponded to 37 kDa, in good agreement with the predicted molecular weight of 34.4 kDa for the 306–amino acid Fstl1 protein.

Fstl1 Prevents NRVMs From Hypoxia/Reoxygenation-Induced Apoptosis Through Activation of Akt and ERK Signaling
There are no reports of Fstl1-induced changes in intracellular signaling. Thus, NRVMs in serum-free media were transduced with Ad-Fstl1, and Western immunoblot analysis of signaling proteins was performed. Adenovirus-mediated overexpression of Fstl1 in NRVMs led to a marked increase in the activating phosphorylation of Akt at Ser473 (Figure 4). Consistent with an increase in Akt signaling, increases in the phosphorylation of mTOR and FOXO proteins, downstream targets of Akt, were also observed. Transduction with Ad-Fstl1 also led to an increase in ERK phosphorylation in NRVMs.

Both Akt and ERK signaling promote cardiomyocyte survival.2,21 Thus, we examined the effect of Fstl1 expression on apoptosis after hypoxia/reoxygenation in NRVMs. Cells were transduced with Fstl1-expressing and control adenoviral vectors and then exposed to media without serum. Parallel

Figure 3. Fstl1 is secreted from cells. A, HEK293 cells were transfected with a plasmid expressing V5 epitope–tagged Fstl1 protein. Western blot analysis using anti-V5 antibody indicates that Fstl1 is present in the cell pellet and the culture media. B, NRVMs were transfected with an adenovirus expressing Fstl1 or β-galactosidase. Western blot analysis using anti-Fstl1 antibody revealed Fstl1 protein expression in the cell pellet and the culture media, and the intensity of these bands increased in cells that were transduced with Ad-Fstl1.

Figure 4. Activation of intracellular signaling pathways by Fstl1. After transfection of Ad-Fstl1, NRVMs were cultured for 36 hours, and cell lysates were prepared for Western blot analysis. Transduction of Ad-Fstl1 led to the activating phosphorylation of Akt1 and 2 and ERK1/2 in NRVMs. Phosphorylation of FOXO1 and 3 and mTOR, downstream targets of Akt signaling, also increased.
cultures were maintained in serum-free media and exposed to normoxia or 12 hours of hypoxia followed by 24 hours of reoxygenation (hypoxiareoxygenation). Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining was performed to evaluate the effect of Fstl1 on cardiac myocyte apoptosis. As shown in Figure 5A, treatment with Ad-Fstl1 reduced the frequency of TUNEL-positive cells under conditions of hypoxiareoxygenation stress. Quantification of TUNEL-positive myocytes revealed that Ad-Fstl1 significantly reduced TUNEL-positive cell number after hypoxiareoxygenation compared with Ad-βgal-transfected cells (*P<0.01 compared with Ad-βgal–transfected NRVMs after hypoxiareoxygenation). To corroborate the effects of Fstl1 on apoptosis, nucleosome fragmentation was assessed by enzyme-linked immunosorbent assay. As shown in Figure 5C, hypoxiareoxygenation-induced nucleosome fragmentation was significantly suppressed by Ad-Fstl1 (*P<0.01 compared with Ad-βgal–transfected NRVMs after hypoxiareoxygenation).

To examine the functional significance of Akt and ERK in Fstl1-mediated cytoprotection, NRVMs were pretreated with specific inhibitors and subjected to hypoxiareoxygenation. Cells were exposed to LY294002 (10 μmol/L), a PI3K inhibitor, or with U0126 (10 μmol/L), an MEK1/2 inhibitor, 3 hours before hypoxiareoxygenation stress. Figure 6A shows representative fluorescent photographs of TUNEL staining for each experimental group. As shown in Figure 6B, transduction with Ad-Fstl1 reduced TUNEL-positive cells after exposure to hypoxiareoxygenation compared with cells treated with Ad-βgal, but the protective action of Ad-Fstl1 was partially attenuated by preincubation with LY294002 or U0126 (*P<0.05). In agreement with these findings, the inhibitory effect of Fstl1 on nucleosome fragmentation was significantly attenuated by treatment with each inhibitor (Figure 6C).

**Fstl1 Functions as an Endogenous Cardiac Myocyte Survival Factor**

To assess the role of endogenous Fstl1, NRVMs were transfected with siRNA targeting Fstl1. Forty-eight hours after transfection, cultures were exposed to hypoxiareoxygenation stress. As shown in Figure 5B, transduction with Ad-Fstl1 reduced TUNEL-positive cell number after hypoxiareoxygenation compared with cells treated with Ad-βgal, but the protective action of Ad-Fstl1 was partially attenuated by preincubation with LY294002 or U0126 (P<0.05). In agreement with these findings, the inhibitory effect of Fstl1 on nucleosome fragmentation was significantly attenuated by treatment with each inhibitor (Figure 6C).
after transfection, both mRNA and protein levels of Fstl1 were significantly attenuated, as shown by QRT-PCR and Western blot analysis (Figure 7A and 7B). Fstl1 protein expression level was decreased by 85% in cell lysate and by 90% in culture media by siRNA treatment (P<0.001). At this time point, nucleosome fragmentation was analyzed in serum-deprived cells. As shown in the Figure 7C, knockdown of Fstl1 resulted in increased apoptosis in normoxic cells as well as cells subjected to hypoxia/reoxygenation stress, suggesting that endogenous Fstl1 plays a role in maintaining cardiomyocyte viability. Fstl1 knockdown resulted in reduced phosphorylation of Akt at baseline but had no effect on baseline ERK signaling (Figure 7D). These Western immunoblot observations were corroborated with a second source of siRNA targeting Fstl1 (Figure 7D).

**Discussion**

In this study, we have found that Fstl1 transcript and protein are upregulated in the myocardium after Akt1 transgene activation. Our studies have also revealed that Fstl1 is a secreted protein and that its overexpression will promote Akt and ERK1/2 signaling in cardiac myocytes and protect cardiac myocytes from hypoxia/reoxygenation-induced apoptosis through activation of these pathways. Conversely, siRNA-mediated knockdown of Fstl1 led to an increase in the frequency of stress-induced apoptosis, and this was associ-
signal is increased when cultures are transduced with the immunodetectable Fstl1 protein in the cell media, and this from cardiac myocytes. Cardiac myocyte cultures display fusion injury, which was accompanied with a reduction in delivery of Fstl1 protected myocardium from ischemia/reperfusion, and permanent LAD ligation. Finally, the systemic ical stimuli including pressure overload, ischemia/reperfusion expression was also upregulated in the heart after patholog-ated with a reduction in basal Akt phosphorylation. Fstl1 expression was also upregulated in the heart after pathologi-cal stimuli including pressure overload, ischemia/reperfusion, and permanent LAD ligation. Finally, the systemic delivery of Fstl1 protected myocardium from ischemia/reperfusion injury, which was accompanied with a reduction in apoptosis.

A number of lines of evidence suggest that Fstl1 is secreted from cardiac myocytes. Cardiac myocyte cultures display immunodetectable Fstl1 protein in the cell media, and this signal is increased when cultures are transduced with the}

**Figure 7.** Knockdown of endogenous Fstl1 increases apoptosis in response to hypoxia/reoxygenation. A, QRT-PCR analysis indicates that transduction of siRNA decreased Fstl1 mRNA by 70% (*P < 0.05) in cultured cardiac myocytes. B, Representative immunoblot analysis of Fstl1 protein expression in cell lysates and culture media of cardiac myocyte cultures treated with siRNA directed to Fstl1 or an unrelated sequence. C, Nucleosome fragmentation assay shows that knockdown of endogenous Fstl1 expression in serum-deprived cardiac myocyte cultures increases apoptosis in cells under normoxic conditions and in cells treated with hypoxia/reoxygenation (H/R) (*P < 0.05). D, Immunoblot analysis reveals that Fstl1 ablation results in decreased Akt phosphorylation, but not that of ERK, in cultured cardiac myocytes. Two commercial sources of siRNA targeting Fstl1 were employed in these assays.

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**The follistatin family of proteins is generally believed to function by binding to and modifying the function of members of the TGF-β superfamily.** Follistatin binds to growth and differentiation factor 8 (GDF8), also referred to as myostatin, as well as activin A and B, and various bone morphogenic proteins (BMP) including BMP-2, -4, and -7. Similarly, Fstl3 binds to a subset of these TGF-β superfamily members including myostatin. Myostatin (GDF8) is of interest because it is expressed by the heart and is reported to have antihypertrophic and antiproliferative actions on cultured cardiac myocytes. However, it is controversial whether myostatin controls heart size in vivo. It is also of interest that GDF15 is upregulated in the heart by pressure overload or ischemic injury, and it exhibits cardioprotective func-tions. It is also reported that serum levels of GDF15 serve as an independent predictor of adverse events in patients with chronic heart failure and acute coronary syndrome. Fin ally, BMP-2 has been reported to act on cardiac myocytes to inhibit apoptosis via the SMAD pathway.

In contrast to the aforementioned considerations, it is not clear whether Fstl1 functions by binding to TGF-β superfamily proteins in a manner similar to follistatin or Fstl3. In this regard, follistatin and Fstl3 display a 29% amino acid sequence identity in mice, but Fstl1 shares only 7% homology with follistatin and 6% homology with Fstl3. Currently, we do not favor the hypothesis that Fstl1 acts indirectly on cardiac myocytes through its ability to bind to TGF-β superfamily protein members. In cell culture experiments, we found that Fstl1 expression activated signaling pathways and promoted cell viability in response to hypoxia/reoxygenation. Because these cell culture experiments were performed in
serum-free media, it is unlikely that Fstl1 alters cellular responses in cardiac myocytes solely through its ability to modulate the function of the binding partner. Another protein with higher homology to Fstl1 than follistatin or Fstl3 is SPARC, which exhibits a 14% sequence identity. Thus, Fstl1 may be functionally more similar to SPARC than to follistatin and Fstl3, and the structural similarity with SPARC suggests that Fstl1 may function to modulate intracellular signaling by controlling cell interactions with extracellular matrix. Additional experiments will be required to elucidate the receptor-mediated signaling events downstream of Fstl1 in the heart.

Adenovirus-mediated Fstl1 overexpression led to the up-regulation of Akt and ERK signaling in cardiac myocytes and improved cardiac myocyte survival. Furthermore, conditioned media from myocytes transduced with Ad-Fstl1 activated Akt signaling and protected against hypoxia/reoxygenation stress–induced apoptosis in cultures of NRVMs (Y. Oshima, MD, PhD, unpublished data, 2008). Because Akt and ERK are involved in cellular survival,2,21,38 it is likely that the cardioprotective actions of exogenous Fstl1 result from the upregulation of these signaling systems. Consistent with this hypothesis, treatment with inhibitors of PI3K or ERK diminished the antiapoptotic activity of exogenous Fstl1. Furthermore, knockdown of endogenous Fstl1 led to a reduction in Akt phosphorylation and an increase in the frequency of myocyte apoptosis. When it is considered that Fstl1 is upregulated in the Akt-activated heart, it appears that Fstl1 could act as a positive feedback loop to promote myocyte survival, or it could act to promote the survival of neighboring myocytes. In this regard, it has been reported that myocyte survival is also modulated by Pim-1, heme oxygenase-1, and hypoxia-inducible factor-1α, which function within the Akt signaling network.39–41 Further studies are needed to assess whether the prosurvival function of secreted Fstl1 is mediated by these other signaling proteins.

In conclusion, these data show for the first time that Fstl1 is secreted by cardiac myocytes and that this factor is upregulated in the heart by an activation of Akt signaling or by injury. Overexpression of Fstl1 is cardioprotective, whereas ablation of Fstl1 leads to an increase in cardiac myocyte apoptosis. We propose that Fstl1 is a cardioprotective factor that is secreted by the heart.

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Disclosures
Nara Bioscience, Inc, has licensed intellectual property related to Fstl1 from Boston University. Dr Walsh is a founder and stockholder of Nara Bioscience. No research funding was provided by Nara Bioscience. The other authors report no conflicts.

References


Clinical Perspective

On injury, the heart secretes factors that influence its function. It is widely recognized that the protein kinase Akt plays an important role in regulating intracellular signaling pathways that control cellular survival and physiological growth. To identify novel factors involved in regulating cardiac function, we sought to isolate and characterize proteins that are secreted from murine heart in response to myocardial Akt activation. The secreted protein follistatin-like 1 (Fstl1) is upregulated in heart in response to transgenic Akt activation as well as ischemia/reperfusion injury, pressure overload, and myocardial infarction. Delivery of the Fstl1 gene to mice protected the heart from ischemia/reperfusion injury and reduced cardiac myocyte death, whereas ablation of Fstl1 expression in myocytes led to an increase in apoptotic cell death in vitro. Thus, Fstl1 functions as an endogenous cardiac myocyte survival factor that is secreted by the heart in response to stress. As such, Fstl1 may serve a useful diagnostic or therapeutic function.
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