The American Journal of Pathology, Vol. 181, No. 6, December 2012

http://dx.doi.org/10.1016/j.ajpath.2012.08.020

Cardiovascular, Pulmonary, and Renal Pathology

Short-Term Akt Activation in Cardiac Muscle Cells Improves Contractile Function in Failing Hearts

Ichiro Shiojima,*† Stephan Schiekofer,‡ Jochen G. Schneider,§ Kurt Belisle,‡ Kaori Sato,* Martin Andrassy,¶ Gennaro Galasso,*‖ and Kenneth Walsh*

From the Molecular Cardiology/Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, Massachusetts; the Department of Cardiovascular Medicine,† Osaka University Graduate School of Medicine, Suita, Japan; the Center of Geriatric Medicine,‡ Bezirksklinikum Regensburg, Regensburg, Germany; the Translational & Experimental Medicine Group,§ Luxembourg Center of Systems Biomedicine, Esch-sur-Alzette, Luxembourg; the Department of Internal Medicine III,¶ Heidelberg University, Heidelberg, Germany; and the Department of Cardiology,‖ Naples University, Naples, Italy*

Akt is a serine/threonine protein kinase that is activated by a variety of growth factors or cytokines in a phosphatidylinositol 3-kinase–dependent manner. By using a conditional transgenic system in which Akt signaling can be turned on or off in the adult heart, we previously showed that short-term Akt activation induces a physiological form of cardiac hypertrophy with enhanced coronary angiogenesis and maintained contractility. Here we tested the hypothesis that induction of physiological hypertrophy by short-term Akt activation might improve contractile function in failing hearts. When Akt signaling transiently was activated in murine hearts with impaired contractility, induced by pressure overload or doxorubicin treatment, contractile dysfunction was attenuated in both cases. Importantly, improvement of contractility was observed before the development of cardiac hypertrophy, indicating that Akt activation improves contractile function independently of its growth-promoting effects. To gain mechanistic insights into Akt-mediated positive inotropic effects, transcriptional profiles in the heart were determined in a pressure overload–induced heart failure model. Biological network analysis of differentially expressed transcripts revealed significant alterations in the expression of genes associated with cell death, and these alterations were reversed by short-term Akt activation. Thus, short-term Akt activation improves contractile function in failing hearts. This beneficial effect of Akt on contractility is hypertrophy-independent and may be mediated in part by inhibition of cell death associated with heart failure. ***(Am J Pathol 2012, 181:1969–1976; http://dx.doi.org/10.1016/j.ajpath.2012.08.020)***

Heart failure is a major cause of mortality and morbidity worldwide.[1–4] One common feature of heart failure is hypertrophy of individual cardiac muscle cells in the myocardium.[5–8] Cardiac hypertrophy observed in patients with hypertension, myocardial infarction, or valvular heart disease is considered to be an adaptive response because hypertrophy can normalize the increase in wall stress induced by mechanical overload. However, increased cardiac mass is clinically associated with increased morbidity and mortality,[9] and sustained overload eventually leads to contractile dysfunction and heart failure.[10–12] Thus, stress-induced or pathologic cardiac hypertrophy appears to be detrimental for the heart. Conversely, normal postnatal growth of the heart or exercise-induced cardiac growth also occurs through hypertrophy of individual cardiac muscle cells. These forms of so-called physiological cardiac hypertrophy are associated with normal or enhanced contractile function and are morphologically and molecularly distinct from stress-induced hypertrophy.[13,14]

Akt is a serine/threonine protein kinase that mediates cellular growth responses in multiple organisms and cell types.[15,16] There are three *Akt* genes in mammalian genomes (*Akt1/PKBα*, *Akt2/PKBβ*, and *Akt3/PKBγ*). Loss of *Akt1* gene in mice leads to general growth retardation and spontaneous apoptosis in restricted cell types.[17,18] *Akt2* gene disruption results in abnormal glucose metabolism and mild growth deficiency,[19,20] whereas *Akt3*

Supported by NIH grants HL102874, AG34972, AG15052, and HL68758 (K.W.), and Landesforschungsschwerpunkt Baden-Württemberg (S.S.).

Accepted for publication August 9, 2012.

I.S. and S.S. contributed equally to this work.

Supplemental material for this article can be found at *http://ajp.amjpathol.org* or at *http://dx.doi.org/10.1016/j.ajpath.2012.08.020*.

Address reprint requests to Stephan Schiekofer, M.D., Center of Geriatric Medicine, Bezirksklinikum Regensburg, Universitätsstr. 84, 93053 Regensburg, Germany. E-mail: stephan.schiekofer@medbo.de.

knockout mice show a selective growth defect of the brain.[21,22] Furthermore, these genes display a considerable degree of functional overlap, exemplified by the combined deletion of *Akt1* and *Akt2* genes, which results in perinatal lethality with severe growth retardation and multiple developmental defects[23] and by *Akt1/Akt3* double-knockout mice, which are embryonic-lethal owing to a placental defect.[24] In the heart, Akt is an important positive regulator of normal postnatal cardiac growth[25] and also is activated by exercise training,[26–28] by pressure overload,[29] and is activated in diseased human hearts.[30] Overexpression of activated Akt1 or Akt3 in the heart is sufficient to induce cardiac hypertrophy and, in some cases, heart failure in transgenic (TG) mice.[31–34] These data suggest that Akt-dependent signaling pathways are involved in both physiologic and pathologic cardiac growth.

By using a conditional TG system, in which the expression of the Akt transgene can be turned on or off at desired time points, we previously showed that short-term Akt activation induces physiologic hypertrophy but long-term Akt activation leads to pathologic hypertrophy and heart failure.[35] In cardiac hypertrophy induced by short-term Akt activation, contractile function was preserved. Because physiologic hypertrophy can be adaptive by reducing wall stress without adverse effects on contractile function, we tested the hypothesis that induction of physiologic hypertrophy by short-term Akt activation might attenuate contractile dysfunction in failing hearts. We show that transient Akt activation improves contractile function in two different heart failure models and we explored the potential mechanisms of Akt-mediated positive inotropic effects by transcriptome analysis.

Materials and Methods

Animals

Generation, genotyping, and the initial characterization of cardiac-specific inducible Akt TG mice have been described previously.[35] In brief, two lines of TG mice (tet-Akt and αMHC-tTA) were crossed to generate double-TG (DTG) mice. When DTG mice are treated with doxycycline (DOX), the expression of Akt transgene is turned off. When DOX is withdrawn, Akt transgene expression is induced specifically in the heart. αMHC-tTA single-TG mice were used as controls and treated with DOX in the same manner as DTG animals.

Heart Failure Models

For pressure overload–induced heart failure models, mice were subjected to ascending aorta constriction (AAC) at the age of 8 weeks as described previously.[36] DOX was withdrawn at the age of 12 weeks (4 weeks after AAC) to induce *Akt* transgene expression in the heart. DOX had been given continuously since before birth. Echocardiography was performed at the age of 8 (before AAC), 12 (4 weeks after AAC), 13 (1 week after *Akt* activation), and 14 weeks (2 weeks after *Akt* activation). For Adriamycin (ADR; Sigma-Aldrich, St. Louis, MO)-induced heart failure models, ADR was administered to 12-week-old mice in 3 equal intraperitoneal injections for 2 weeks (8 mg/kg for a single injection, cumulative dose of 24 mg/kg body weight). DOX was withdrawn at the age of 16 weeks (4 weeks after initial ADR injection) to induce *Akt* transgene in the heart. DOX had been given continuously since before birth. Echocardiography was performed at the age of 12 (before ADR injection), 16 (4 weeks after initial ADR injection), 17 (1 week after *Akt* activation), and 18 weeks (2 weeks after *Akt* activation).

Echocardiography

Transthoracic echocardiography was performed with an ACUSON 256 sector scanner (Siemens, Mountain View, CA) equipped with a 13-MHz transducer as described previously.[35]

Microarray Analysis

Transcriptional profiles were determined in pressure overload–induced heart failure models. Mice were sacrificed at predetermined time points. Hearts were excised and snap-frozen in liquid nitrogen. RNA extraction, cDNA synthesis, biotinylated cRNA preparation, and hybridization of cRNA to microarrays (GeneChip Mouse Expression Set 430; Affymetrix, Santa Clara, CA) were performed as described.[37–39] Data were obtained at three time points [before AAC, 4 weeks after AAC, and 6 weeks after AAC (2 weeks after *Akt* activation)], and four independent sets of hybridizations were performed at each time point. An automated analysis of related transcripts was performed using Ingenuity Pathways Analysis (Redwood City, CA), a web-delivered application that evaluates biological networks. Data analysis was performed as described.[37–39] In addition, automated analysis of groups of biologically related genes was performed using GenMAPP and MAPPFinder version 2.0 (*http://www.genmapp.org*) as described.[37] MAPPFinder is a tool that integrates the annotations of the Gene Ontology Project (*http://www.geneontology.org*) with the free software package Gen-MAPP. MAPPFinder identifies Gene Ontology Project terms with overrepresented numbers of gene-expression charges. GenMAPP generates graphic files within which gene expression data can be viewed in the context of the biological knowledge contained in the Gene Ontology Project database.

qPCR Analysis

Expression levels of selected transcripts were examined by quantitative real-time PCR (qPCR) as described.[37] The primers in Table 1 were used for qPCR analysis.

Table 1. qPCR Analysis. Expression levels of selected transcripts were examined by qPCR as described.[37]

Transcript	Forward primer	Reverse primer
Actin α 1	5′-TGCGCGACATCAAAGAGAAG-3′	5′-ACCGATAAAGGAAGGCTGGAA-3′
ATP-binding cassette, sub-family A (ABC1), member 4	5′-GTCCTCAGTTTGATGCAATCGA-3′	5′-CAGGCGGTCAGCGTAGAGA-3′
BCL2-like 11	5′-GCCCCTACCTCCCTACAGACA-3′	5′-CCGCAGCTCCTGTGCAAT-3′
Bone morphogenetic protein 8b	5′-GCCCCTCGAACAGCAAGAC-3′	5′-TGCCTGCGGCAAACTTCT-3′
Calsequestrin 1	5′-CAAGGTGGCAAAGAAGCTGACT-3′	5′-CCTCCACGAAGCTCACAATCTC-3′
Carboxylesterase 3	5′-CAGCCTGTGGCTGTTTTCCT-3′	5′-CCTGCCCTCCAACAGCAT-3′
Cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	5′-GATGCCCAAGACTAGCCTGAAGT-3′	5′-GGTTTCCCCGTGGCTTTG-3′
Cullin 5	5′-AGACTCCAGGACAGTGCAATGA-3′	5′-CCTCTCTGTTGAGTCCAAGTATGC-3′
Cyclin B2	5′-CACTTTAGCCAAGTACCTGATGGA-3′	5′-GCCTGTGTAATACTGCTGCTTCA-3′
Cytokine receptor-like factor 1	5′-TCACCACCAGCTCTCAAGGATT-3′	5′-GCACTTGGACGAAGTAAACG-3′
Fast skeletal myosin alkali light chain	5′-ACAAGGACCAGGGAGGTTATGA-3′	5′-CCAGAGTGGCGAGGACATG-3′
Fibroblast growth factor 4	5′-CTACCTGCTGGGCCTCAAAA-3′	5′-GTAAAGAAAGGCACACCGAAGAG-3′
Fibrillin 1	5′-GCCCTGCTGGACACAAATTTAA-3′	5′-CCGTTTGCCAGAGCTGTGTA-3′
Follistatin related protein	5′-CAAGTGCCTCAACCCATCCT-3′	5′-TCCTGTCTTCTCCTCCTCTGTGT-3′
Gap junction membrane channel protein α1	5′-CAGCCTGAGTGCGGTCTACAC-3′	5′-AAGGACACCACCAGCATGAAG-3′
Glucosaminyl (*N*-acetyl) transferase 1, core 2	5′-GCTGGAGATGATCCTTACAGCAA-3′	5′-GGCACAGTCACGGGTCATG-3′
Histocompatibility 2, L region	5′-CGACGGCTGCGATTACATC-3′	5′-CGTTCCCGTTCTTCAGGTATCT-3′
Insulin-like growth factor binding protein 1	5′-CCGCGGATGAGCTTTCTG-3′	5′-ATTTCTTGAGGTCGGCGATCT-3′
Insulin-like growth factor binding protein 5	5′-GAAGGACCGCAGAAAGAAGCT-3′	5′-GTTCGGATTCCTGTCTCATCTCA-3′
Mitogen activated protein kinase 13	5′-ACTGGCTCACCCCTTCTTTGA-3′	5′-CCACGCTGAGTTTCTCATGTTC-3′
Myomesin 2	5′-CCTGCCTATGACCTGACGTTCT-3′	5′-TGGCATTCACTGCTCGAATTC-3′
Myotrophin	5′-GCTGTCTATGAGGGTCATGTTTCC-3′	5′-GCTTTGATTGCCTGGTTGTCA-3′
Ninjurin 1	5′-GGTGGAGCAGGGCAATGA-3′	5′-TGACCACGACGATGATGAAAAC-3′
Phospholipase A2, group IIC	5′-CCTCCACCCTCAGCAGTTTCT-3′	5′-TCACCGTCCCATTGACAATG-3′
Procollagen, type V, α 1	5′-GATGGCATCCGAGGTCTGAA-3′	5′-CACGCCAAGCTTTCCCTTT-3′
Procollagen, type VIII, α 1	5′-CCAGGGAGAGTATCTGCCAGATA-3′	5′-AAGGTACAGTCAGCTCGGCAGTA-3′
Prolactin receptor	5′-GCCTTCCACATCCCTGAGATC-3′	5′-CATCGGCAATGCTGTGGTAA-3′
Prostaglandin D2 synthase	5′-TCCGGGAGAAGAAAGCTGTATT-3′	5′-CTGGTTTTTCCTGAGGAAGGTAGAG-3′
SRY-box containing gene 4	5′-CAAGCGGCTAGGCAAACG-3′	5′-GTTGCCCGACTTCACCTTCTT-3′
Tenascin C	5′-ACAGCTACCGACGGGATCTTC-3′	5′-TTGTCAACTTCCGGTTCAGCTT-3′
Thrombospondin 1	5′-CAACGTCCTTCTTACCCTTGACA-3′	5′-CCACAGATAGCTTGGAGGTCCTT-3′
Thymoma viral proto-oncogene 1	5′-CCTTCCTTACGGCCCTCAA-3′	5′-ACACAATCTCCGCACCATAGAA-3′
Zinc finger protein 352	5′-GCCATTGGTTTCCATTTTGG-3′	5′-AAGTCTCCCTGGTGTCAACTCTTG-3′

Results

Improvement of Contractile Function by Transient Akt Activation

To examine the effects of inducible *Akt* activation in failing hearts, mice were subjected to AAC at the age of 8 weeks and *Akt* transgene was induced by withdrawing DOX at the age of 12 weeks, when contractile dysfunction was apparent by echocardiography (Figure 1). At the age of 13 weeks (1 week after *Akt* activation), there was a significant increase in the percentage of fractional shortening in DTG animals compared with control animals. This improvement of contractility was maintained at 14 weeks of age (2 weeks after *Akt* activation). Of note, there was a significant increase in posterior wall thickness in DTG mice compared with control animals at 14 weeks old but not at 13 weeks old. This is consistent with our previous observation that a significant increase in heart weight starts to be observed at day 10 after *Akt* transgene induction.[35] These findings indicate that transient *Akt* activation in failing hearts leads to improvement of contractile function, and this positive inotropic effect of *Akt* activation is independent of its growth-promoting effect.

To examine whether this beneficial effect of *Akt* on contractility is observed in a different form of heart failure, *Akt* transiently was activated in failing hearts induced by ADR treatment. Mice received three injections of ADR once a week beginning at the age of 12 weeks. *Akt* transgene was induced by withdrawing DOX at the age of 16 weeks (Figure 2). At this time point, contractile dysfunction was apparent by echocardiography. As was the case with pressure overload–induced heart failure, transient *Akt* activation in cardiac muscle cells improved ADR-induced contractile function, and this beneficial effect of *Akt* was independent of its growth-promoting effect because there was a significant increase in the percentage of fractional shortening at the age of 17 weeks (1 week after *Akt* activation) when there was no increase in wall thickness. Taken together, *Akt* activation improves contractile function in two different models of heart failure independently of its growth-promoting effect on the myocardium.

Microarray Analysis of Differentially Expressed Genes

To gain insights into *Akt*-mediated, positive inotropic effects, transcriptional profiles in the heart were determined in a pressure overload–induced heart failure model. RNA samples were prepared at 8 weeks (time point 1: before AAC), 12 weeks (time point 2: 4 weeks after AAC when there is apparent contractile dysfunction), and 14 weeks

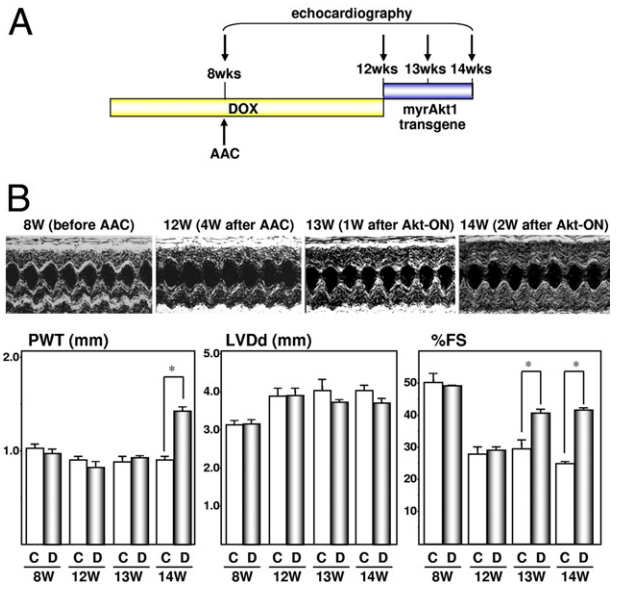

Figure 1. Short-term *Akt* activation improves contractile function in pressure overload–induced heart failure. **A:** Temporal profile of DOX treatment, AAC operation, and echocardiography. **B:** Echocardiography. **Top panels:** representative M-mode recordings; **bottom panels:** posterior wall thickness (PWT), left ventricular end-diastolic dimension (LVDd), and percentage fractional shortening (%FS). C, control animals; D, DTG animals. $^{*}P < 0.01$.

(time point 3: 2 weeks after *Akt* activation, when contractile function has been improved), and transcriptome analysis was performed. When time points 1 and 2 were compared, 381 genes differentially were regulated, of which 212 were up-regulated and 169 were down-regulated. When time points 2 and 3 were compared, 328 genes differentially were regulated, of which 162 genes

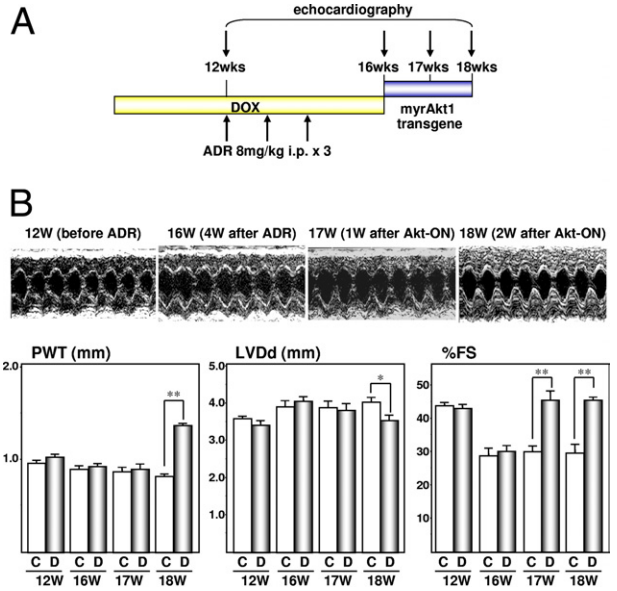

Figure 2. Short-term *Akt* activation improves contractile function in ADR-induced heart failure. **A:** Temporal profile of DOX treatment, ADR treatment, and echocardiography. **B:** Echocardiography. **Top panels:** representative M-mode recordings; **bottom panels:** posterior wall thickness (PWT), left ventricular end-diastolic dimension (LVDd), and percentage fractional shortening (%FS). C, control animals; D, DTG animals. $^{*}P < 0.05$, $^{**}P < 0.01$.

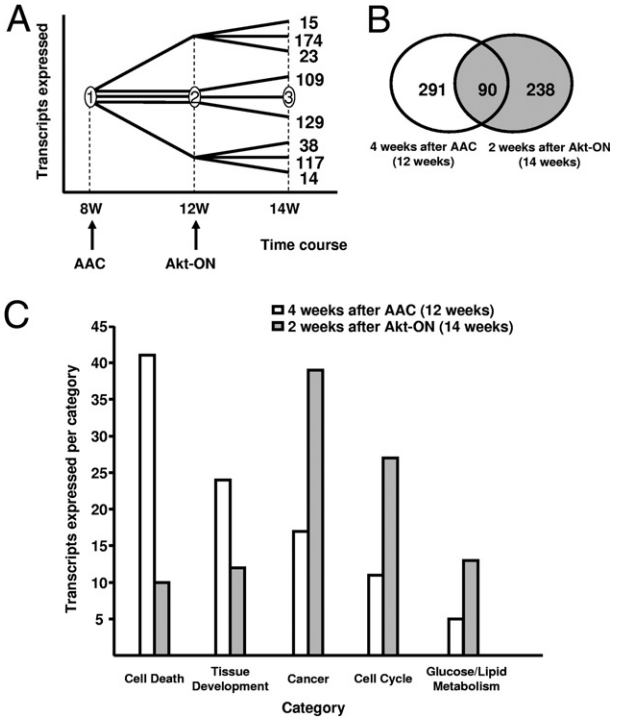

Figure 3. Transcriptome analysis of DTG hearts after AAC and *Akt* activation. **A:** Temporal profile evaluation of transcripts that are expressed differentially at time points 4 weeks after AAC (12 weeks) and 2 weeks after Akt-ON (14 weeks). **B:** Venn diagram representing the sets of transcripts that are regulated differentially 4 weeks after AAC (12 weeks) and 2 weeks after Akt-ON (14 weeks). **C:** Categorization of differentially expressed transcripts 4 weeks after AAC (12 weeks) and 2 weeks after Akt-ON (14 weeks) revealed by Ingenuity.

were up-regulated and 166 genes were down-regulated (Figure 3A). Venn diagram analysis revealed that the expression of 619 transcripts differentially were regulated, of which 291 genes differentially were regulated only when heart failure was developing, 238 genes differentially were regulated only when heart failure was improving, and 90 genes differentially were regulated during both of these processes (Figure 3B). Differential expression of genes with a fold change more than +2.0 or less than −2.0 with a *P* value less than 0.01 were confirmed by qPCR and are listed in Tables 2 and 3. Categorization of differentially regulated genes using Gene Ontology Analysis revealed that genes associated with cell death and tissue development were up-regulated during heart failure progression, which was attenuated by *Akt* activation. Conversely, genes associated with cancer, carbohydrate metabolism, and cell cycle were up-regulated when heart failure was improving by *Akt* activation (Figure 3C). Biological network analysis of differentially expressed transcripts performed using Ingenuity Pathways Analysis revealed a set of genes, categorized as cell "death network," with significant alterations in the expression levels at 12 weeks of age, 4 weeks after AAC, when there is apparent contractile dysfunction (see Supplemental Figure S1 at *http://ajp.amjpathol.org*). After 2 weeks of *Akt* activation, these alterations in the expression levels of a specific set of genes were attenuated or in some cases reversed (see Supplemental Figure S2 at

Table 2. Summary of Transcripts Identified by Microarray and Confirmed by qPCR as Differentially Regulated Between 8 Weeks (Before AAC) and 12 Weeks (4 Weeks After AAC)

Transcript name	Fold change (microarrays), $P < 0.01$	Fold change (qPCR), $P < 0.01$	Accession code	Transcript symbol
Histocompatibility 2, L region	+16.2	+12.2	BC023409	*H2-L*
Glucosaminyl (*N*-acetyl) transferase 1, core 2	+4.7	+3.7	NM173442	*Gcnt1*
Ninjurin 1	+3.7	+2.0	NM013610	*Ninj1*
Actin α1	+2.4	+2.0	NM009606	*Acta1*
Cullin 5	+2.1	+2.1	NM027807	*Cul5*
Phospholipase A2, group IIC	−2.1	−4.7	NM008868	*Pla2g2c*
BCL2-like 11	−2.2	−2.4	NM009754	*Bcl2l11*
Carboxylesterase 3	−2.5	−3.0	NM053200	*Ces3*
Fibroblast growth factor 4	−2.6	−3.1	NM010202	*Fgf4*
Zinc finger protein 352	−3.1	−2.5	NM153102	*Zfp352*
Bone morphogenetic protein 8b	−3.7	−4.4	NM007559	*Bmp8b*
Insulin-like growth factor binding protein 1	−4.3	−5.9	NM008341	*Igfbp1*
SRY-box containing gene 4	−4.9	−4.0	NM009238	*Sox4*
ATP-binding cassette, subfamily A (ABC1), member 4	−28.8	−15.8	NM007378	*Abca4*

http://ajp.amjpathol.org). Biological network analysis also identified a network of genes categorized as an "Akt pathway" that differentially are regulated 2 weeks after *Akt* activation, which support the authenticity of this type of analysis (see Supplemental Figures S3 and S4 at *http://ajp.amjpathol.org*). Names and symbols of the transcripts that are regulated differentially in Supplemental Figures S1 and S2 and in Supplemental Figures S3 and S4 are summarized in Supplemental Table S1 (available at *http://ajp.amjpathol.org*). Taken together, categorization and biological network analysis suggest that alterations in the expression levels of genes associated with cell death may be involved in the positive inotropic effects of *Akt* activation in the setting of heart failure.

Discussion

Unlike pathologic cardiac hypertrophy observed in patients with hypertension or valvular heart diseases, physiologic cardiac hypertrophy as observed in trained athletes or during normal postnatal growth is associated with maintained or enhanced contractile function.

By using an inducible TG system in which cardiac Akt signaling can be turned on or off at desired time points, we previously showed that short-term *Akt* activation in the adult heart induces a physiologic form of cardiac hypertrophy.[35] Because cardiac hypertrophy can be beneficial for the heart through reduction of wall stress, we hypothesized that physiologic hypertrophy induced by short-term *Akt* activation might improve contractile function in

Table 3. Summary of Transcripts Identified by Microarray and Confirmed by qPCR as Differentially Regulated Between 12 Weeks (4 Weeks After AAC) and 14 Weeks (2 Weeks After Akt-ON)

Transcript name	Fold change (microarrays), $P < 0.01$	Fold change (qPCR), $P < 0.01$	Accession code	Transcript symbol
ATP-binding cassette, subfamily A (ABC1), member 4	+13.8	+11.4	NM007378	*Abca4*
Mitogen-activated protein kinase 13	+9.7	+10.8	NM011950	*Mapk13*
Cyclin B2	+7.1	+5.5	NM007630	*Ccnb2*
Tenascin C	+6.5	+4.8	NM011607	*Tnc*
Cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	+6.1	+6.5	NM173385	*Cilp*
Thymoma viral proto-oncogene 1	+4.2	+6.6	NM009652	*Akt1*
Procollagen, type V, α1	+3.5	+4.6	NM015734	*Col5a1*
Insulin-like growth factor binding protein 5	+3.0	+2.1	NM010518	*Igfbp5*
Follistatin-related protein	+2.9	+1.9	NM008047	*Fstl*
Bone morphogenetic protein 8b	+2.8	+3.1	NM007559	*Bmp8b*
Thrombospondin 1	+2.7	+3.0	NM011580	*Thbs1*
Cytokine receptor-like factor 1	+2.7	+3.1	NM018827	*Crlf1*
Calsequestrin 1	+2.5	+3.5	NM009813	*Casq1*
Zinc finger protein 352	+2.3	+2.9	NM153102	*Zfp352*
Fast skeletal myosin alkali light chain	+2.2	+2.4	NM021285	*Myl1*
Procollagen, type VIII, alpha 1	+2.2	+2.8	NM007739	*Col8a1*
Fibrillin 1	+2.1	+2.6	NM007993	*Fbn1*
Myotrophin	+2.1	+2.0	NM008098	*Mtpn*
Gap junction membrane channel protein α 1	−2.4	−5.5	NM010288	*Gja1*
Prostaglandin D2 synthase	−3.2	−5.2	NM008963	*Ptgds*
Carboxylesterase 3	−5.2	−3.2	NM053200	*Ces3*
Glucosaminyl (*N*-acetyl) transferase 1, core 2	−5.6	−6.6	NM173442	*Gcnt1*
Myomesin 2	−10.8	−17.0	NM008664	*Myom2*

failing hearts. To test this hypothesis, we used two different murine heart failure models (pressure overload– or ADR-induced heart failure models) and showed that transient *Akt* activation after the establishment of heart failure improved contractility in both cases. This experiment is relevant because it mimics the clinical situation in which therapeutic intervention is started after the onset of heart failure, and has been difficult to perform with conventional knockout or TG mice in which deletion or overexpression of a specific gene is initiated during early embryogenesis. It was rather unexpected, however, that the transient *Akt* activation attenuated contractile dysfunction at earlier time points when no apparent hypertrophy was observed. This clearly indicates that the beneficial effect of *Akt* on contractility is independent of its effect to promote physiologic cardiac hypertrophy. Thus, although transient *Akt* activation does improve contractile function in the failing heart, this improvement is not caused by a reduction in wall stress by Akt-mediated physiologic hypertrophy. Of note, short-term *Akt* activation does not affect contractile function when *Akt* is turned on in normal hearts,[35] indicating that the beneficial effect of Akt is specific to failing hearts. This raises the question as to the mechanism by which transient Akt activation has positive inotropic effects in the setting of heart failure.

Previous studies have associated *Akt* activation with pathologic cardiac hypertrophy. It recently was shown in the canine congestive heart failure/cardiac recovery model that phosphatidylinositol 3Kγ and phosphatidylinositol 3Kα expressions increase during the congestive heart failure and cardiac recovery phases, respectively.[40] The study went on to extrapolate that, based on the measured increase of phosphatidylinositol 3Kα/Akt during the experimental recovery phase, the phosphatidylinositol 3Kα/Akt signaling pathway potentially plays a role in compensatory cardiac hypertrophy. However, this and other studies linking Akt and pathologic cardiac hypertrophy generally were based on constitutively active forms of the Akt pathway. In contrast, our model indicates that contractile improvements after heart failure may not be completely linked with hypertrophic growth, as evidenced by the lack of hypertrophy (heart rate/body weight) seen in our model. We do recognize, however, that any direct comparison of experimental results must be viewed with caution because of differences in the experimental models. Therefore, echocardiography was chosen as an outcome marker because it is a well-established technique for measuring cardiac function and thus allows our work to be compared directly with previous works in which cardiac recovery was associated with hypertrophy.

In our previous study, we showed that short-term *Akt* activation enhances vascular endothelial growth factor secretion from the myocardium and promotes coronary angiogenesis, which is required for the maintenance of cardiac function during Akt-induced physiologic heart growth: blocking the vascular endothelial growth factor signal during short-term *Akt* activation results in early transition from physiologic to pathologic hypertrophy and contractile dysfunction.[35] It also has been shown that myocardial capillary density is decreased in hearts subjected to pressure overload.[41] Therefore, one possible explanation for the Akt-mediated positive inotropic effect is that Akt induces vascular endothelial growth factor expression in cardiac muscle cells, thereby increasing the otherwise reduced capillary density in failing hearts. A second possibility is that Akt modulates Ca^{2+} handling in cardiac myocytes and enhances contractility. It has been shown that Akt increases the protein expression levels of sarcoplasmic reticulum calcium ATPase 2 (SERCA2) in TG mice overexpressing constitutively active Akt[42] or in rat hearts infected with Akt adenoviruses.[43] Because overexpression of SERCA2 has been shown to improve contractile function in heart failure models,[44] Akt may show positive inotropic effects by increasing the expression levels of SERCA2. Previous investigations,[45] among others, have shown a link between activation of the phosphatidylinositol 3K/Akt pathway and SERCA2a regulation. Although the scope of our investigations did not specifically include measurements of SERCA2a or other calcium-regulating proteins, it seems more than likely that in light of the aforementioned investigations, Akt-dependent regulation of SERCA2a is a potential mechanism for affecting myocyte contractility. Moreover, the potential mechanisms responsible for our model's improved cardiac contractility will be the subject of our next series of investigations. A third possibility suggested by our transcriptome analysis is that Akt improves contractile function by suppressing cell death in the failing heart. Several lines of evidence support the notion of a causal role of apoptosis in heart failure. It was shown that even low levels of cardiomyocyte apoptosis, induced by procaspase-8, led to lethal dilated cardiomyopathy in TG mice, and that this phenotype was prevented by treatment with caspase inhibitor.[46] Caspase inhibitor treatment also ameliorated the development of heart failure in Gαq TG mice.[47] Moreover, loss of gp130-mediated cell survival signals led to early transition from adaptive cardiac growth to heart failure in response to pressure overload.[48] Given that Akt strongly promotes cell survival through phosphorylation of multiple downstream targets including forkhead transcription factor or BCL-2 antagonist of cell death,[49] improved contractile function by inducible expression of Akt in the heart may be owing in part to its prosurvival effects. Collectively, transient activation of Akt signaling in cardiac muscle cells attenuates contractile dysfunction in the failing heart, presumably through multiple mechanisms including modulation of coronary angiogenesis, Ca^{2+} handling, and cell survival.

In the clinical setting, Akt is not a suitable therapeutic target for patients with heart failure because sustained activation of Akt is deleterious for the heart, presumably through induction of extensive cardiac hypertrophy.[31,35] Because the positive inotropic effects of Akt can be separated from its growth-promoting effects, downstream substrates of Akt kinase that mediate its cardioprotective effects may be promising therapeutic targets for heart failure.

References

1. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, Zheng ZJ, Flegal K, O'Donnell C, Kittner S, Lloyd-Jones D, Goff DC Jr, Hong Y, Adams R, Friday G, Furie K, Gorelick P, Kissela B, Marler J, Meigs J, Roger V, Sidney S, Sorlie P, Steinberger J, Wasserthiel-Smoller S, Wilson M, Wolf P: Heart disease and stroke statistics–2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2006, 113:e85–e151
2. Braunwald E, Colucci W, Grossman W: Clinical aspects of heart failure: high-output heart failure; pulmonary edema. In: Heart disease: a textbook of cardiovascular medicine. Edited by E. Braunwald. Philadelphia, W.B. Sanders, 1997, pp 445-470
3. Harjola VP, Follath F, Nieminen MS, Brutsaert D, Dickstein K, Drexler H, Hochadel M, Komajda M, Lopez-Sendon JL, Ponikowski P, Tavazzi L: Characteristics, outcomes, and predictors of mortality at 3 months and 1 year in patients hospitalized for acute heart failure. Eur J Heart Fail 2010, 12:239–248
4. Lam CS, Donal E, Kraigher-Krainer E, Vasan RS: Epidemiology and clinical course of heart failure with preserved ejection fraction. Eur J Heart Fail 2011, 13:18–28
5. Olivetti G, Capasso JM, Meggs LG, Sonnenblick EH, Anversa P: Cellular basis of chronic ventricular remodeling after myocardial infarction in rats. Circ Res 1991, 68:856–869
6. Gerdes AM, Kellerman SE, Moore JA, Muffly KE, Clark LC, Reaves PY, Malec KB, McKeown PP, Schocken DD: Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. Circulation 1992, 86:426–430
7. Barry SP, Davidson SM, Townsend PA: Molecular regulation of cardiac hypertrophy. Int J Biochem Cell Biol 2008, 40:2023–2039
8. Toischer K, Rokita AG, Unsold B, Zhu W, Kararigas G, Sossalla S, Reuter SP, Becker A, Teucher N, Seidler T, Grebe C, Preuss L, Gupta SN, Schmidt K, Lehnart SE, Kruger M, Linke WA, Backs J, Regitz-Zagrosek V, Schafer K, Field LJ, Maier LS, Hasenfuss G: Differential cardiac remodeling in preload versus afterload. Circulation 2010, 122:993–1003
9. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP: Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 1990, 322: 1561–1566
10. MacLellan WR, Schneider MD: Genetic dissection of cardiac growth control pathways. Annu Rev Physiol 2000, 62:289–319
11. Molkentin J, Dorn IG: Cytoplasmic signaling pathways that regulate cardiac hypertrophy. Annu Rev Physiol 2001, 63:391–426
12. Frey N, Olson EN: Cardiac hypertrophy: the good, the bad, and the ugly. Annu Rev Physiol 2003, 65:45–79
13. Hudlicka O, Brown MD: Postnatal growth of the heart and its blood vessels. J Vasc Res 1996, 33:266–287
14. Richey PA, Brown SP: Pathological versus physiological left ventricular hypertrophy: a review. J Sports Sci 1998, 16:129–141
15. Shiojima I, Walsh K: Role of Akt signaling in vascular homeostasis and angiogenesis. Circ Res 2002, 90:1243–1250
16. Shiojima I, Walsh K: Regulation of cardiac growth and coronary angiogenesis by the Akt/PKB signaling pathway. Genes Dev 2006, 20:3347–3365
17. Chen WS, Xu PZ, Gottlob K, Chen ML, Sokol K, Shiyanova T, Roninson I, Weng W, Suzuki R, Tobe K, Kadowaki T, Hay N: Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. Genes Dev 2001, 15:2203–2208
18. Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ: Akt1/pkbalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 2001, 276:38349–38352
19. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB 3rd, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ: Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 2001, 292:1728–1731
20. Garofalo RS, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL, Coskran T, Black SC, Brees DJ, Wicks JR, McNeish JD, Coleman KG: Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. J Clin Invest 2003, 112:197–208
21. Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T, Efstratiadis A, Birnbaum MJ: Role for Akt3/protein kinase Bgamma in attainment of normal brain size. Mol Cell Biol 2005, 25:1869–1878
22. Tschopp O, Yang ZZ, Brodbeck D, Dummler BA, Hemmings-Mieszczak M, Watanabe T, Michaelis T, Frahm J, Hemmings BA: Essential role of protein kinase B gamma (PKB gamma/Akt3) in postnatal brain development but not in glucose homeostasis. Development 2005, 132:2943–2954
23. Peng XD, Xu PZ, Chen ML, Hahn-Windgassen A, Skeen J, Jacobs J, Sundararajan D, Chen WS, Crawford SE, Coleman KG, Hay N: Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. Genes Dev 2003, 17:1352–1365
24. Yang ZZ, Tschopp O, Di-Poi N, Bruder E, Baudry A, Dummler B, Wahli W, Hemmings BA: Dosage-dependent effects of Akt1/protein kinase Balpha (PKBalpha) and Akt3/PKBgamma on thymus, skin, and cardiovascular and nervous system development in mice. Mol Cell Biol 2005, 25:10407–10418
25. Shiojima I, Yefremashvili M, Luo Z, Kureishi Y, Takahashi A, Tao J, Rosenzweig A, Kahn CR, Abel ED, Walsh K: Akt signaling mediates postnatal heart growth in response to insulin and nutritional status. J Biol Chem 2002, 277:37670–37677
26. Wilkins BJ, Dai YS, Bueno OF, Parsons SA, Xu J, Plank DM, Jones F, Kimball TR, Molkentin JD: Calcineurin/NFAT coupling participates in pathological, but not physiological, cardiac hypertrophy. Circ Res 2004, 94:110–118
27. Konhilas JP, Maass AH, Luckey SW, Stauffer BL, Olson EN, Leinwand LA: Sex modifies exercise and cardiac adaptation in mice. Am J Physiol Heart Circ Physiol 2004, 287:H2768–H2776
28. Konhilas JP, Widegren U, Allen DL, Paul AC, Cleary A, Leinwand LA: Loaded wheel running and muscle adaptation in the mouse. Am J Physiol Heart Circ Physiol 2005, 289:H455–H465
29. Naga Prasad SV, Esposito G, Mao L, Koch WJ, Rockman HA: Gbetagamma-dependent phosphoinositide 3-kinase activation in hearts with in vivo pressure overload hypertrophy. J Biol Chem 2000, 275:4693–4698
30. Haq S, Choukroun G, Lim H, Tymitz KM, del Monte F, Gwathmey J, Grazette L, Michael A, Hajjar R, Force T, Molkentin JD: Differential activation of signal transduction pathways in human hearts with hypertrophy versus advanced heart failure. Circulation 2001, 103:670–677
31. Shioi T, McMullen JR, Kang PM, Douglas PS, Obata T, Franke TF, Cantley LC, Izumo S: Akt/protein kinase B promotes organ growth in transgenic mice. Mol Cell Biol 2002, 22:2799–2809
32. Matsui T, Li L, Wu JC, Cook SA, Nagoshi T, Picard MH, Liao R, Rosenzweig A: Phenotypic spectrum caused by transgenic overexpression of activated Akt in the heart. J Biol Chem 2002, 277:22896–22901
33. Condorelli G, Drusco A, Stassi G, Bellacosa A, Roncarati R, Iaccarino G, Russo MA, Gu Y, Dalton N, Chung C, Latronico MV, Napoli C, Sadoshima J, Croce CM, Ross J Jr: Akt induces enhanced myocardial contractility and cell size in vivo in transgenic mice. Proc Natl Acad Sci U S A 2002, 99:12333–12338
34. Taniyama Y, Ito M, Sato K, Kuester C, Veit K, Tremp G, Liao R, Colucci WS, Ivashchenko Y, Walsh K, Shiojima I: Akt3 overexpression in the heart results in progression from adaptive to maladaptive hypertrophy. J Mol Cell Cardiol 2005, 38:375–385
35. Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, Colucci WS, Walsh K: Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. J Clin Invest 2005, 115:2108–2118
36. Liao R, Jain M, Cui L, D'Agostino J, Aiello F, Luptak I, Ngoy S, Mortensen RM, Tian R: Cardiac-specific overexpression of GLUT1 prevents the development of heart failure attributable to pressure overload in mice. Circulation 2002, 106:2125–2131
37. Schiekofer S, Galasso G, Sato K, Kraus BJ, Walsh K: Impaired revascularization in a mouse model of type 2 diabetes is associated with dysregulation of a complex angiogenic-regulatory network. Arterioscler Thromb Vasc Biol 2005, 25:1603–1609
38. Schiekofer S, Belisle K, Galasso G, Schneider JG, Boehm BO, Burster T, Schmitz G, Walsh K: Angiogenic-regulatory network revealed by molecular profiling heart tissue following Akt1 induction in endothelial cells. Angiogenesis 2008, 11:289–299
39. Schiekofer S, Shiojima I, Sato K, Galasso G, Oshima Y, Walsh K: Microarray analysis of Akt1 activation in transgenic mouse hearts

reveals transcript expression profiles associated with compensatory hypertrophy and failure. Physiol Genomics 2006, 27:156–170
40. Braz JC, Gill RM, Corbly AK, Jones BD, Jin N, Vlahos CJ, Wu Q, Shen W: Selective activation of PI3Kalpha/Akt/GSK-3beta signalling and cardiac compensatory hypertrophy during recovery from heart failure. Eur J Heart Fail 2009, 11:739–748
41. Izumiya Y, Shiojima I, Sato K, Sawyer DB, Colucci WS, Walsh K: VEGF blockade promotes the transition from compensated cardiac hypertrophy to failure in response to pressure overload. Hypertension 2006, 47:887–893
42. Kim YK, Kim SJ, Yatani A, Huang Y, Castelli G, Vatner DE, Liu J, Zhang Q, Diaz G, Zieba R, Thaisz J, Drusco A, Croce C, Sadoshima J, Condorelli G, Vatner SF: Mechanism of enhanced cardiac function in mice with hypertrophy induced by overexpressed Akt. J Biol Chem 2003, 278:47622–47628
43. Cittadini A, Monti MG, Iaccarino G, Di Rella F, Tsichlis PN, Di Gianni A, Stromer H, Sorriento D, Peschle C, Trimarco B, Sacca L, Condorelli G: Adenoviral gene transfer of Akt enhances myocardial contractility and intracellular calcium handling. Gene Ther 2006, 13:8–19
44. del Monte F, Hajjar RJ: Targeting calcium cycling proteins in heart failure through gene transfer. J Physiol 2003, 546:49–61
45. Kim SJ, Abdellatif M, Koul S, Crystal GJ: Chronic treatment with insulin-like growth factor I enhances myocyte contraction by upregulation of Akt-SERCA2a signaling pathway. Am J Physiol Heart Circ Physiol 2008, 295:H130–H135
46. Wencker D, Chandra M, Nguyen K, Miao W, Garantziotis S, Factor SM, Shirani J, Armstrong RC, Kitsis RN: A mechanistic role for cardiac myocyte apoptosis in heart failure. J Clin Invest 2003, 111:1497–1504
47. Hayakawa Y, Chandra M, Miao W, Shirani J, Brown JH, Dorn GW 2nd, Armstrong RC, Kitsis RN: Inhibition of cardiac myocyte apoptosis improves cardiac function and abolishes mortality in the peripartum cardiomyopathy of Galpha (q) transgenic mice, Circulation 2003, 108:3036–3041
48. Hirota H, Chen J, Betz UA, Rajewsky K, Gu Y, Ross J Jr, Muller W, Chien KR: Loss of a gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress. Cell 1999, 97:189–198
49. Datta SR, Brunet A, Greenberg ME: Cellular survival: a play in three Akts. Genes Dev 1999, 13:2905–2927