REVIEW

Regulation of cardiac growth and coronary angiogenesis by the Akt/PKB signaling pathway

Ichiro Shiojima^{1,2,4} and Kenneth Walsh^{1,3}

¹Molecular Cardiology, Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, Massachusetts 02118, USA; ²Department of Cardiovascular Science and Medicine, Chiba University School of Medicine, Chiba 260-8670, Japan

Postnatal growth of the heart is primarily achieved through hypertrophy of individual myocytes. Cardiac growth observed in athletes represents adaptive or physiological hypertrophy, whereas cardiac growth observed in patients with hypertension or valvular heart diseases is called maladaptive or pathological hypertrophy. These two types of hypertrophy are morphologically, functionally, and molecularly distinct from each other. The serine/threonine protein kinase Akt is activated by various extracellular stimuli in a phosphatidylinositol-3 kinase-dependent manner and regulates multiple aspects of cellular functions including survival, growth and metabolism. In this review we will discuss the role of the Akt signaling pathway in the heart, focusing on the regulation of cardiac growth, contractile function, and coronary angiogenesis. How this signaling pathway contributes to the development of physiological/pathological hypertrophy and heart failure will also be discussed.

Growth of the heart during embryonic development occurs primarily through proliferation of cardiac myocytes. However, cardiac myocytes withdraw from the cell cycle soon after birth, and subsequent growth of the heart during postnatal development is achieved predominantly through hypertrophy rather than hyperplasia of individual myocytes (Pasumarthi and Field 2002; Olson and Schneider 2003). In fact, there is almost a threefold increase in cardiac myocyte diameter in humans during development from infants to adults (Hudlicka and Brown 1996). Cardiac growth during normal postnatal development or the hypertrophy observed in trained athletes is referred to as "physiological," and it is characterized by normal or enhanced contractile function and normal architecture and organization of cardiac structure (Richey and Brown 1998). On the other hand, cardiac hypertrophy is also observed in patients with pathological condi-

tions such as hypertension, myocardial infarction, and valvular heart diseases. This type of cardiac growth is called "pathological" hypertrophy, and is frequently associated with contractile dysfunction, interstitial fibrosis, and re-expression of fetal-type cardiac genes such as atrial natriuretic peptide and β -myosin heavy chain (Molkentin and Dorn 2001; Frey and Olson 2003). These data indicate that pathological cardiac hypertrophy is morphologically and molecularly distinct from physiological cardiac hypertrophy. The pathological form of cardiac hypertrophy is initially recognized as an adaptive response to increased external load, because increased wall stress induced by overload is attenuated by the increase in wall thickness. However, increased cardiac mass is clinically associated with increased morbidity and mortality (Levy et al. 1990), and a sustained overload eventually leads to contractile dysfunction and heart failure through poorly understood mechanisms (Molkentin and Dorn 2001; Frey and Olson 2003). In addition, hypertrophy of individual myocyte is a common feature of failing myocardium (Gerdes et al. 1992). Thus, pathological cardiac hypertrophy appears to be detrimental for the heart when this condition is maintained chronically. These observations lead to the question: What determines the difference between physiological versus pathological cardiac hypertrophy? A related question is: What are the mechanisms by which sustained overload results in heart failure? Answering these questions is important because it will lead to a better understanding of the pathogenesis of heart failure, a leading cause of mortality worldwide (Braunwald and Bristow 2000; Thom et al. 2006).

The serine/threonine protein kinase Akt is an important mediator of phosphatidylinositol-3 kinase (PI3K) signaling and regulates a wide variety of cellular functions in different tissues. Numerous studies have implicated Akt-dependent signaling pathways in the regulation of cardiac growth, contractile function, and coronary angiogenesis. In this review we summarize recent findings on Akt signaling pathway and discuss its role in the development of physiological hypertrophy, pathological hypertrophy, and heart failure.

[Keywords: Akt; hypertrophy; heart failure; angiogenesis; mTOR]
Corresponding authors.

³E-MAIL kxwalsh@bu.edu; FAX (617) 414-2391.

⁴E-MAIL shiojima@faculty.chiba-u.jp; FAX 81-43-226-2979.

Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.1492806>.

Regulation of Akt kinase activity and downstream Akt substrates (Fig. 1)

Mammalian genomes contain three *Akt* genes—*Akt1/PKB α* , *Akt2/PKB β* , and *Akt3/PKB γ* —whereas flies and worms contain one and two *Akt* genes, respectively (Datta et al. 1999; Scheid and Woodgett 2001). These genes encode proteins containing a pleckstrin-homology (PH) domain in the N terminus, a central kinase domain, and a C-terminal hydrophobic motif (Fig. 1). In unstimulated cells, Akt resides in the cytosol and its kinase domain is thought to be masked by C-terminal hydrophobic motif. In response to growth factor stimulation, Akt is recruited to the plasma membrane via its N-terminal PH domain. Membrane-associated Akt is sequentially phosphorylated at its two regulatory phosphorylation sites, T308 and S473 (in the case of Akt1), by upstream kinases called phosphoinositide-dependent protein kinase-1 (PDK1) and the “hydrophobic motif kinase,” respectively (Brazil et al. 2004; Altomare and Testa 2005; Woodgett 2005). Although PDK1 was molecularly identified and characterized as a kinase that phosphorylates a subgroup of AGC (cAMP dependent, cGMP dependent, and protein kinase C) family protein kinases, the nature of the “hydrophobic motif kinase” has been elusive. Previous studies have implicated several proteins as potential S473 kinases including PDK1, Akt (via autophosphorylation), MAPKAP kinase-2, integrin-linked kinase, protein kinase C (PKC) isoforms, ATM kinase, and double-stranded DNA-dependent protein kinase (Dong and Liu 2005). However, recent evidences strongly sug-

gest that the rapamycin-insensitive complex of mammalian target of rapamycin (mTORC2) containing mTOR, mLST8/G β L, and rictor (rapamycin-insensitive companion of mTOR), is responsible for growth factor-induced S473 phosphorylation (Sarbasov et al. 2005b). Akt is fully active when these two regulatory sites are phosphorylated, and it then translocates to various sites within the cell and exerts its biological effects by phosphorylating downstream substrates. Akt is then dephosphorylated and inactivated by protein phosphatases. Protein phosphatase 2A (PP2A) is a T308 phosphatase and PH domain leucine-rich repeat protein phosphatase (PHLPP) predominantly dephosphorylates S473 (Andjelkovic et al. 1996; Gao et al. 2005). Akt kinase activity is also regulated both positively and negatively by its interacting proteins, including T-cell leukemia/lymphoma 1 (TCL1), a *Drosophila* Tribbles homolog 3 (TRB3), and C-terminal modulator protein (CTMP) (Brazil et al. 2002; Du and Tsichlis 2005).

A variety of downstream Akt substrates have been identified, and the list continues to grow in number. Bad, caspase-9, I κ B-kinase, and FOXO mediate the anti-apoptosis effects of Akt, and progression of cell cycle by Akt is mediated by Akt-dependent phosphorylation of substrates including MDM2, p21, p27, and Myt1 (Brazil et al. 2004). Akt also regulates glucose metabolism by phosphorylating glycogen synthase kinase-3 (GSK-3) and AS160 (Akt substrate of 160 kDa) (Dugani and Klip 2005; Woodgett 2005), and protein synthesis and cell growth is mediated by phosphorylation of tuberous sclerosis complex 2 (TSC2) (Hay and Sonenberg 2004). Some of these substrates actually mediate multiple actions of Akt. For example, FOXO regulates cell cycle progression as well as cell survival, and GSK-3 is implicated in both cell cycle and protein synthesis regulation. Cell type-specific Akt substrates also exist such as endothelial nitric oxide synthase (eNOS), which regulates nitric oxide biosynthesis in endothelial cells (Shiojima and Walsh 2002). Thus, it is likely that multiple Akt substrates regulate various aspects of cellular responses in a context-dependent manner.

Figure 1. Regulation of Akt kinase activity and downstream Akt substrates. Upon insulin/IGF stimulation, Akt is recruited to the plasma membrane via its N-terminal PH domain, and activated by phosphorylation at T308 (by PDK1) and S473 (by mTORC2). Active Akt translocates to various sites within the cell and phosphorylates downstream substrates. Akt kinase activity is then down-regulated by dephosphorylation of the two regulatory sites by protein phosphatases (T308 by PP2A, and S473 by PHLPP).

Akt knockout/transgenic studies (general information)

Physiological roles of Akt in vivo have been investigated using knockout (KO) or transgenic (TG) mice. Targeted disruption of the *Akt1* gene in mice results in a growth retardation phenotype (Chen et al. 2001; H. Cho et al. 2001b). *Akt2* KO mice exhibit insulin resistance and, depending on genetic background, mild growth retardation (H. Cho et al. 2001a; Garofalo et al. 2003). In this regard, a mutation in the human *AKT2* gene has also been reported that produces a dominant-negative mutant of AKT2 protein and is associated with severe insulin resistance and diabetes (George et al. 2004). In contrast, *Akt3* KO mice do not show the general growth retardation phenotype, but brain size is reduced in adult *Akt3* KO mice due to the reduction in both cell size and cell number (Easton et al. 2005; Tschopp et al. 2005). Functional redundancy among the three *Akt* gene products is demonstrated by mutant mice with combined deletions

of these genes. *Akt1/Akt2* double-KO animals die shortly after birth due to defects in multiple tissues including skeletal muscle, bone, and skin, and *Akt1/Akt3* double-mutant mice die in utero (Peng et al. 2003; Yang et al. 2005). Thus, the phenotypes of these compound mutants are more severe than the sum of single-KO phenotype, suggesting a high degree of functional compensation among the three *Akt* genes in mammals.

In transgenic studies, myristoylated Akt1 (membrane-targeted Akt1) or a phosphorylation mimicking mutant of Akt1 (Akt1 T308D/S473D) has been used as a constitutively active Akt1. Kinase activity of these mutants is insensitive to PI3K inhibition. Although attachment of myristoylated sequence generally results in membrane localization of the protein, it has been shown that myristoylated Akt1 also localizes to the nucleus and cytoplasm (Adini et al. 2003). Akt1 E40K mutant, which exhibits enhanced basal activity and marginally enhanced response to growth factor stimulation, is also used as an active Akt1 mutant. Basal and growth factor-stimulated activity of Akt1 E40K is attenuated by PI3K inhibition (Bellacosa et al. 1998), and enhanced basal activity is presumably due to increased affinity of mutant PH domain to PI3K-generated phospholipids. Overexpression of Akt1 in pancreatic β cells leads to both hypertrophy and hyperplasia of these cell types (Bernal-Mizrachi et al. 2001; Tuttle et al. 2001), consistent with the idea that Akt promotes cell growth and proliferation. Expression of Akt1 in T lymphocytes results in the development of lymphomas (Malstrom et al. 2001; Rathmell et al. 2003), and prostate cancer formation is observed in TG mice overexpressing Akt1 in prostate epithelial cells (Majumder et al. 2003). However, Akt1 overexpression in mammary glands fails to induce tumor formation (Hutchinson et al. 2001; Schwertfeger et al. 2001, 2003; Ackler et al. 2002). Thus, sustained Akt activation is sufficient to induce malignant transformation in some, but not all cell types. Endothelial cell-specific overexpression of Akt1 results in embryonic lethality, associated with abnormal vascular remodeling and patterning (Sun et al. 2005). Overexpression of Akt1 in endothelial cells in the adult stage also induces pathological blood vessel formation reminiscent of tumor vasculature (Phung et al. 2006). However, during blood flow cessation-induced neointimal formation, brief Akt activation in endothelial cells attenuates lesion formation (Mukai et al. 2006). Presumably, transient activation of Akt1 in endothelial cells results in the maintenance of a functionally intact endothelial layer, and this pacifies the stimuli that promote smooth muscle growth in this model. Furthermore, ischemia or vascular endothelial growth factor (VEGF)-induced angiogenesis and mobilization of endothelial progenitor cells are impaired in Akt1 KO mice (Ackah et al. 2005), and new vessels that form in response to ischemic injury are immature and leaky in Akt1 KO mice (Chen et al. 2005). These data suggest that Akt signaling plays an important role in maintaining vascular homeostasis, and that fine-tune control of the Akt signal in endothelial cells is required for normal vascular patterning and remodeling.

Regulation of cardiomyocyte differentiation and embryonic heart growth by Akt signaling

Initial specification and differentiation of embryonic cardiomyocytes in the anterior lateral mesoderm are regulated by multiple signaling pathways including those activated by bone morphogenetic proteins (BMPs) and Wnts (Olson and Schneider 2003). It was initially reported that inhibition of PI3K attenuates cardiomyocyte differentiation in embryonic stem (ES) cells, suggesting that PI3K pathway is essential for cardiomyocyte differentiation (Klinz et al. 1999). Using P19CL6, a clonal derivative of P19 teratocarcinoma cell line that differentiate into cardiomyocytes in response to DMSO treatment, it was reported that Akt is activated during cardiomyocyte differentiation, and that treatment with PI3K or Akt inhibitors results in attenuation of P19CL6 differentiation into cardiomyocytes (Naito et al. 2003). PI3K inhibition in DMSO-treated P19CL6 cells is associated with down-regulation of early cardiac marker genes such as *Nkx-2.5* and *GATA-4*, suggesting that the PI3K–Akt pathway is critical during early-stage cardiomyocyte differentiation. Furthermore, it was shown that inhibition of PI3K/Akt pathway in the early stage results in the suppression of Wnt/ β -catenin signaling (Naito et al. 2005). Although Wnt signaling is inhibitory to cardiogenesis in chick and frog embryos (Marvin et al. 2001; Schneider and Mercola 2001; Tzahor and Lassar 2001), early Wnt signaling during P19CL6 differentiation is essential for cardiomyogenesis (Nakamura et al. 2003; Naito et al. 2005), the latter observation presumably reflecting the role of Wnt pathway in mesoderm patterning and/or early cardiac specification. Thus, the PI3K/Akt pathway may promote cardiomyogenesis by maintaining the activity of Wnt signaling. Similar synergism between the PI3K/Akt pathway and the Wnt/ β -catenin pathway has been reported in PC12 cells (Fukumoto et al. 2001) and endothelial cells (Skurk et al. 2005b). Alternatively, Akt may promote cardiomyocyte differentiation by positively regulating the transcriptional activity of *GATA-4* via inactivation of GSK3- β (Morisco et al. 2001), or Akt may enhance BMP signaling—as shown in osteoblasts (Ghosh-Choudhury et al. 2002)—that is essential for the expression of the cardiac transcription factor *Nkx-2.5* (Olson and Schneider 2003). More simply, the PI3K/Akt pathway may enhance cardiomyocyte differentiation by promoting survival of differentiated myocytes (Matsui et al. 1999; Fujio et al. 2000).

After specification and differentiation of precursor cells in anterior lateral mesoderm into a cardiac lineage, heart growth during embryonic development is attributable to the proliferation of embryonic cardiomyocytes as well as migration and differentiation of progenitors derived from the secondary heart field (Pasumarthi and Field 2002; Olson and Schneider 2003; Buckingham et al. 2005). Proliferation of embryonic cardiomyocytes are regulated by multiple growth factors including neuregulin from the endocardium (Garratt et al. 2003), BMP10 expressed in the trabecular myocardium (Chen et al. 2004), and retinoic acid and erythropoietin derived from

the epicardium (Stuckmann et al. 2003). It has been shown that human ES cell-derived cardiomyocytes are highly proliferative under serum free conditions, but that PI3K or Akt inhibitors attenuate cardiomyocyte proliferation, suggesting that PI3K/Akt signaling promotes embryonic cardiomyocyte proliferation (McDevitt et al. 2005). However, it should be noted that mouse ES cell-derived cardiomyocytes have little proliferative capacity in vitro, and the precise role of PI3K/Akt signaling in embryonic heart growth needs to be examined in vivo.

Regulation of postnatal cardiac growth by components of the Akt signaling pathway

Genetic studies have shown that the insulin/insulin-like growth factor (IGF)–PI3K–Akt–mTOR–S6K pathway regulates organ growth and body size in flies and mice (Stocker and Hafen 2000; Kozma and Thomas 2002). TG and KO mouse studies have also shown that this same pathway regulates heart size and cardiac function.

Insulin/IGF

Insulin and IGF are potent activators of Akt in cardiac myocytes. IGF signaling regulates both embryonic and postnatal body/organ size, as evidenced by general growth retardation in IGF-I or type 1 IGF receptor (IGF1R) KO mice (Efstratiadis 1998). In the heart, increased cardiac IGF-I production was shown to be associated with physiological cardiac hypertrophy in athletes (Neri Serneri et al. 2001), suggesting that cardiac IGF signaling promotes the physiological form of cardiac growth. However, results of TG mouse studies in which IGF-I or IGF1R is overexpressed in the heart are somewhat conflicting. Overexpression of IGF-I in the heart using rat α -myosin heavy chain promoter results in myocyte proliferation (Reiss et al. 1996), whereas α -skeletal actin promoter-mediated overexpression of IGF-I in cardiac and skeletal muscle induces physiological cardiac hypertrophy in the early phases of postnatal development and pathological hypertrophy in later phases (DeLaughter et al. 1999). IGF1R overexpression in the heart using mouse α -myosin heavy chain promoter results in physiological hypertrophy associated with increased myofiber size, enhanced contractile function, and activation of the PI3K–Akt–S6K pathway (McMullen et al. 2004b). Although the reason for such discrepancies are unknown, these conflicting results are likely to be due to different expression levels of transgene products and/or different promoters used in these TG studies.

Insulin signaling is an important regulator of glucose metabolism in vertebrates, and the heart is an insulin-responsive organ. Insulin promotes glucose uptake, glycolysis, glucose oxidation, and glycogen synthesis in the heart (An and Rodrigues 2006). Under basal conditions, fatty acids are preferred to glucose as a source of ATP generation, and insulin modifies this fuel hierarchy both by stimulating glucose consumption and by inhibiting fatty acid utilization (Hue et al. 2002). In addition to its

well-known metabolic effects, it has been shown that insulin also regulates postnatal cardiac growth and function. Cardiomyocyte-restricted insulin receptor (IR) KO mice exhibit a decrease in heart size and impaired contractile function (Belke et al. 2002), and this small heart phenotype is mediated by attenuated Akt signaling in the heart of IR KO mice (Shiojima et al. 2002). However, in response to pathological hypertrophic stimuli such as pressure overload or isoproterenol infusion, heart size increases in both wild-type and KO mice with similar final heart weight, although the heart size of IR KO mice at baseline is smaller than that of wild-type animals (Hu et al. 2003). Thus, intrinsic insulin signaling in the heart is required for normal physiological cardiac growth during postnatal development, but is dispensable for, and may attenuate the development of, pathological hypertrophy.

Heart weight is tightly coupled with body weight from infants to adults, and heart size is also extremely sensitive to nutritional status: Heart size is reduced in starved subjects or in patients with anorexia nervosa (Keys et al. 1950; de Simone et al. 1994), and increased in obese subjects without hypertension or other cardiovascular and/or metabolic abnormalities (Crisostomo et al. 1999). Consistently, cardiac Akt activity in mice is up-regulated during rapid postnatal growth, and down-regulated by overnight fasting (Shiojima et al. 2002). Presumably, postnatal heart growth is coordinately regulated by both IGF-I and insulin signaling, the former primarily reflecting the intrinsic activity of the hypothalamic–pituitary axis, whereas the latter reflects the external nutritional condition, and these two signaling pathways converge upstream of Akt to regulate overall heart size.

PI3K/PTEN (Fig. 2)

Class I PI3Ks are heterodimeric enzymes composed of a regulatory subunit and a catalytic subunit that are sub-

Figure 2. Regulation of Akt activity in the heart by PI3K and PTEN. During normal growth of the heart, class Ia PI3K (PI3K α and PI3K β) is activated by receptor tyrosine kinases and positively regulates cardiac growth predominantly via Akt-dependent pathway. On the other hand, class Ib PI3K (PI3K γ) is activated by GPCR through G $\beta\gamma$ subunits and negatively regulates contractility in an Akt-independent manner. Lipid phosphatase PTEN antagonizes both class Ia and class Ib PI3K. However, during pathological heart growth, PI3K γ activates Akt and stimulates cardiac growth. Akt may also modulate contractile function, when activated either by class Ia or class Ib PI3K.

divided into two subgroups: class Ia PI3Ks (PI3K α , PI3K β , PI3K δ) and a class Ib PI3K (PI3K γ). The regulatory subunits of class Ia PI3Ks include p85 α and its truncated splice variants p50 α and p55 α , as well as p85 β and p55 γ . The catalytic subunits of class Ia PI3Ks include p110 α , p110 β , and p110 δ . Class Ib PI3K, PI3K γ , is composed of a regulatory subunit p101 and a catalytic subunit p110 γ (Taniguchi et al. 2006). In the heart, p85 α , p85 β , and p101 isoforms and p110 α , p110 β , and p110 γ isoforms are the predominantly expressed regulatory and catalytic subunits, respectively. The distinction between class Ia and class Ib PI3Ks are based on the observations that class Ia PI3Ks are activated by receptor tyrosine kinases and cytokine receptors, whereas class Ib PI3K is activated by G-protein-coupled receptors through G $\beta\gamma$ subunits (Oudit et al. 2004).

Class Ia PI3K is implicated in body/organ size regulation in *Drosophila* (Stocker and Hafen 2000), and the regulatory role of Class Ia PI3K in heart size determination is shown by TG mouse studies: Overexpression of a constitutively active or a dominant-negative form of p110 α in the heart results in an increase or a decrease in heart size, respectively (Shioi et al. 2000). An increase or a decrease in heart size is associated with increased or decreased myocyte size and Akt activity, respectively, and there are no detectable features of heart pathology in these TG lines. Moreover, physiological hypertrophy induced by exercise training or IGF1R overexpression is completely abolished by coexpression of dominant-negative p110 α , whereas pressure overload induces a similar level of hypertrophy in wild-type and dominant-negative p110 α TG animals (McMullen et al. 2003, 2004b). Simultaneous deletion of p85 α and p85 β in the heart also results in a decrease in heart size and impaired growth response to exercise training (Luo et al. 2005). These data suggest that the insulin/IGF-PI3K α/β -Akt pathway is a critical regulator of normal physiological cardiac growth but is dispensable for the development of pathological cardiac hypertrophy.

PTEN is a lipid phosphatase that predominantly dephosphorylates PtdIns(3,4,5)P $_3$ to generate PtdIns(4,5)P $_2$, and therefore acts as a PI3K antagonist. In *Drosophila*, loss of PTEN results in enhanced growth, whereas PTEN overexpression leads to a decrease in cell number and cell size (Stocker and Hafen 2000). In mice, deletion of PTEN in cardiac muscle cells results in cardiac hypertrophy associated with an increase in individual myocyte size and Akt/S6K activity, which is reversed by coexpression of dominant-negative p110 α but not by simultaneous deletion of p110 γ (Crackower et al. 2002). These results are consistent with the notion that PTEN loss results in increased class Ia PI3K activity, leading to cardiac hypertrophy. Furthermore, PTEN deletion in the heart is associated with decreased contractility, which is rescued by simultaneous deletion of p110 γ but not by coexpression of dominant-negative p110 α . At baseline, p110 γ mutant mice exhibit hypercontractility of the heart with normal heart weight and Akt activity, and simultaneous deletion of PTEN and p110 γ in cardiac muscle cells results in cardiac hypertrophy associated

with hypercontractility (Crackower et al. 2002). These observations indicate that PTEN antagonizes both class Ia and class Ib PI3Ks in the heart, and that these two pathways are genetically uncoupled: Class Ia PI3K positively regulates heart size via the Akt pathway, whereas class Ib PI3K negatively regulates contractile function. However, it should be noted that in conditions where pathological hypertrophy is induced, class Ib PI3K (PI3K γ) is selectively up-regulated in the heart and Akt is activated exclusively via PI3K γ isoform (Naga Prasad et al. 2000; Patrucco et al. 2004), suggesting that the GPCR-PI3K γ -Akt axis also contributes to the increase in heart size under conditions that promote pathological hypertrophy.

PDK1

PDK1 was identified as a protein kinase that phosphorylates T308 of Akt1 following activation of PI3K. Subsequently, it was found that PDK1 phosphorylates Ser/Thr residues in the activation loop (T-loop) of a subgroup of the AGC family protein kinases including Akt, S6K, serum- and glucocorticoid-induced protein kinase (SGK), and atypical PKCs (Mora et al. 2004). PDK1 appears to be constitutively active, and both PDK1 and Akt have a PH domain that efficiently interacts with PtdIns(3,4,5)P $_3$. Thus, PtdIns(3,4,5)P $_3$ production following PI3K activation leads to colocalization of these two enzymes at the plasma membrane and results in activation of Akt through PDK1-mediated T-loop phosphorylation (Woodgett 2005).

Studies in *Drosophila* and mice demonstrated that PDK1 regulates body and organ size (K.S. Cho et al. 2001; Rintelen et al. 2001; Lawlor et al. 2002), and the role of PDK1 in the regulation of cardiac growth was demonstrated by PDK1 deletion in cardiac muscle cells in mice. Heart size of mutant mice is smaller than that of wild-type animals due to a decrease in cardiomyocyte cell size, and these animals exhibit dilated cardiomyopathy-like heart failure phenotype (Mora et al. 2003). Furthermore, insulin fails to induce the phosphorylation of Akt and S6K at their T-loop phosphorylation sites in the hearts of mutant mice. Thus, PDK1 is essential for normal heart growth, and is also required to maintain contractile function during postnatal development. It should be noted that the phenotypes of cardiac PDK1 KO mice and dominant-negative p110 α TG mice are quite different in terms of contractile function: PDK1 mutant mice exhibit extensive heart failure, whereas contractile function is maintained in the heart of dominant-negative p110 α TG mice. Although the precise reason is unclear at present, the difference in phenotype may be due to functional ablation of other AGC kinases that are regulated by PDK1 in a PI3K-independent manner. Such PDK1-dependent but PI3K-independent activation of S6K has been demonstrated in *Drosophila* (Radimerski et al. 2002).

Akt

Numerous lines of evidence show that Akt controls heart size. A number of groups have also reported that

overexpression of activated form of Akt1 or Akt3 in the heart under the control of mouse α -myosin heavy chain promoter induces cardiac hypertrophy (Condorelli et al. 2002; Matsui et al. 2002; Shioi et al. 2002; Taniyama et al. 2005). In these studies Akt-induced cardiac hypertrophy is associated with an increase in cardiomyocyte cell size and activation of downstream Akt targets including S6K, GSK-3, and FOXO. However, there also exist some differences in the phenotypes of animals in these studies. Overexpression of E40K mutant of Akt1 results in mild hypertrophy (~40% increase in heart size) and enhanced contractility without signs of cardiac pathology (Condorelli et al. 2002), which can be described as a “physiological” form of cardiac hypertrophy. In contrast, overexpression of myristoylated or phosphomimetic forms of Akt1 leads to a massive increase in heart size (more than twofold increase) associated with impaired contractile function and interstitial fibrosis (Matsui et al. 2002; Shioi et al. 2002), a typical feature of “pathological” cardiac hypertrophy. TG mice expressing myristoylated Akt3 in the heart exhibit cardiac growth that resembles compensated hypertrophy at 4 wk of age but pathological hypertrophy at later ages (Taniyama et al. 2005). In another study using cardiac-specific inducible Akt1 TG mice, it was shown that short-term Akt activation induces “physiological” hypertrophy with a moderate (~80%) increase in heart size, whereas prolonged Akt activation results in “pathological” hypertrophy with a massive (2.7-fold) increase in heart size (Shiojima et al. 2005). The “physiological” nature of cardiac hypertrophy induced by short-term Akt activation is demonstrated by the following observations: (1) preserved contractile function, (2) lack of interstitial fibrosis, (3) lack of fetal cardiac gene induction, and (4) completely reversible hypertrophy. However, pathological cardiac hypertrophy after long-term Akt activation is associated with interstitial fibrosis, induction of fetal genes, LV dilatation, and contractile dysfunction. These data indicate that, although physiological hypertrophy and pathological hypertrophy are morphologically and molecularly quite distinct (Schiekofer et al. 2006), these two forms of cardiac hypertrophy can be induced in a sequential manner by activating a single signaling pathway in the heart. They also suggest that the differences between physiological and pathological hypertrophy may be determined by the overall extent of hypertrophy, which reflects the level and duration of Akt signaling.

In addition to the extent of Akt activation, subcellular distribution of Akt activity might be a determinant of cardiac phenotype. In epithelial cells, nuclear Akt activity is linked to tumorigenesis (Trotman et al. 2006), and IGF-I overexpression in the heart results in nuclear accumulation of phosphorylated Akt (Camper-Kirby et al. 2001). Transgenic overexpression of a nuclear-targeted (nonactivated) form of Akt1 in the heart does not alter the overall heart size but results in an increase in cell number as well as a decrease in cell size due to enhanced cell cycle progression. Nuclear Akt overexpression is also associated with increased contractile function and protection from apoptotic stimuli, and does not lead to

cardiac hypertrophy or cardiomyopathy (Shiraishi et al. 2004; Rota et al. 2005). Thus, regulation of heart size by Akt may be dependent on its targets in the cytosol or plasma membrane.

Whether Akt is required for cardiac growth was examined using Akt1 or Akt2 KO mice. The development of exercise-induced physiological hypertrophy is blunted and contractile function is impaired in Akt1 KO animals after exercise training. In contrast, Akt1 KO mice exhibit enhanced cardiac growth and attenuated contractility in response to pathological growth stimuli (pressure overload) (DeBosch et al. 2006a). Thus, Akt1 is a positive and a negative regulator of physiological and pathological cardiac hypertrophy, respectively, and is required for the maintenance of contractile function in response to both physiological and pathological growth stimuli. On the other hand, Akt2 KO mice exhibit normal cardiac growth in response to pressure overload, and IGF-I- or ET-1-treated Akt2^{-/-} cardiac myocytes display comparable hypertrophic responses to wild-type myocytes. However, insulin-induced glucose uptake is impaired in Akt2^{-/-} myocytes but not in Akt1^{-/-} cells (DeBosch et al. 2006b). Thus, Akt2 predominantly regulates cardiac glucose metabolism but has little impact on cardiac growth control.

GSK-3 (Fig. 3)

GSK-3 was initially identified as a kinase that phosphorylates and inactivates glycogen synthase, but is now recognized as a regulator of multiple processes including development, tumorigenesis, and metabolism (Doble and Woodgett 2003). GSK-3 is constitutively active under unstimulated conditions, whereas insulin signaling down-regulates GSK-3 activity through Akt-mediated N-terminal phosphorylation at Ser21 (GSK-3 α) or Ser9 (GSK-3 β) (Doble and Woodgett 2003). Both in cultured cardiac myocytes and in the heart in vivo, it was shown that hypertrophic stimuli such as ET-1 or pressure overload inhibits GSK-3 activity (Haq et al. 2000), and a constitutively active form of GSK-3 (GSK-3 β /S9A) attenuates heart growth in response to pressure overload or chronic β -adrenergic stimulation (Antos et al. 2002). Transgenic overexpression of wild-type GSK-3 β in the heart also induces an ~30% decrease in heart size (Michael et al. 2004), collectively suggesting that GSK-3 negatively regulates cardiac growth in response to developmental cues as well as pathological stimuli.

Several potential mechanisms are proposed for the negative regulatory role of GSK-3 in cardiac hypertrophy. First, GSK-3 may attenuate cardiac hypertrophy by inducing nuclear export of nuclear factor of activated T cells (NFAT) transcription factors. Because the calcium-dependent phosphatase calcineurin is an inducer of hypertrophy through dephosphorylation and nuclear import of NFAT (Heineke and Molkentin 2006), GSK-3-mediated phosphorylation of NFAT antagonizes the hypertrophic action of calcineurin. Indeed, constitutively active GSK-3 inhibits ET-1-induced NFAT nuclear import (Haq et al. 2000). Second, because GSK-3 induces

Figure 3. Akt-dependent signaling pathways during cardiac growth. GSK-3, TSC2, and FOXO transcription factors are three major substrates of Akt that mediate growth-promoting signals. GSK-3 attenuates the activity of specific transcription factors that mediate hypertrophic responses and inhibits translation by phosphorylating eIF2B ϵ . Phosphorylation of GSK-3 by Akt results in down-regulation of GSK-3 activity and therefore promotes hypertrophy. TSC1/2 complex attenuates mTOR activity through inhibition of small G protein Rheb, and phosphorylation of TSC2 by Akt results in down-regulation of the GAP activity of TSC1/2 and up-regulation of mTOR. The rapamycin-sensitive mTOR complex mTORC1 regulates translation (via S6K and/or 4E-BP1) and coronary angiogenesis (possibly via HIF-dependent VEGF and Ang-2 induction), whereas rapamycin-insensitive mTORC2 regulates actin reorganization and Akt phosphorylation at S473. FOXO transcription factors mediate protein degradation and atrophy of cardiac muscle, and Akt-mediated phosphorylation of FOXO results in inhibition of FOXO activity and FOXO-mediated protein degradation. Thus, Akt regulates protein synthesis, protein degradation, transcription, and angiogenesis during cardiac growth.

nuclear export of GATA-4 and reduces transcriptional activity of myocardin (Morisco et al. 2001; Badorff et al. 2005), both of which are transcription factors implicated in hypertrophic responses of myocytes (Perrino and Rockman 2006; Pipes et al. 2006), GSK-3 may attenuate cardiac hypertrophy by suppressing the activities of these transcription factors. Third, GSK-3 inhibits initiation of protein translation by negatively regulating eukaryotic translation initiation factor 2B ϵ (eIF2B ϵ). Binding of eIF2 to the activated initiator tRNA and formation of a complex with the 40S ribosomal subunit are essential for the initiation of translation (Proud 2005). eIF2B ϵ is one of the subunits of eIF2B, and its activity is negatively regulated through phosphorylation by upstream kinases including GSK-3 (Proud 2005). In cultured cardiac myocytes, it was shown that GSK-3 phosphorylates eIF2B ϵ , and this phosphorylation is critical for anti-hypertrophic effects of GSK-3 (Hardt et al. 2004). eIF2B ϵ phosphorylation is also increased in the heart of GSK-3 β TG mice (Michael et al. 2004). Finally, it was proposed that GSK-3 inhibition induces cardiac hypertrophy through modulation of β -catenin-dependent transcription. GSK-3 is a key component of Wnt signaling pathway. Under basal condition, GSK-3 phosphorylates

β -catenin in a β -catenin degradation complex composed of Axin, adenomatous polyposis coli (APC), GSK-3, and β -catenin, leading to degradation of β -catenin and inhibition of transcription induced by β -catenin and T-cell factor (TCF)/lymphoid enhancer factor (Lef). Upon Wnt stimulation, β -catenin phosphorylation is inhibited, resulting in translocation of β -catenin to the nucleus and activation of β -catenin-dependent gene transcription (Moon et al. 2004). Hypertrophic stimuli induce β -catenin stabilization and enhance β -catenin-dependent gene transcription leading to hypertrophy, whereas dominant-negative Lef1 attenuates agonist-induced hypertrophy (Haq et al. 2003). Interestingly, hypertrophic stimuli lead to recruitment of activated Akt to β -catenin degradation complex, resulting in phosphorylation of GSK-3 β at Ser9 (Haq et al. 2003), suggesting that N-terminal serine phosphorylation of GSK-3 by Akt induces down-regulation of GSK-3 kinase activity toward β -catenin. Previous studies have shown that insulin and the Wnt signaling pathway leading to GSK-3 are insulated from each other: Insulin stimulation does not induce β -catenin stabilization, and Wnt stimulation is not associated with GSK-3 β Ser9 phosphorylation nor activation of glycogen synthase (Brazil et al. 2002). Thus, the mechanism by which hypertrophic stimuli induces β -catenin stabilization through the Akt/GSK-3-dependent pathway in cardiac myocytes appears to be distinct from that of canonical Wnt and insulin signaling pathways.

mTOR (Fig. 3)

mTOR was identified as a mammalian counterpart of yeast TORs, which are the target of the drug rapamycin (Sarbasov et al. 2005a; Wullschleger et al. 2006). Rapamycin forms a complex with FKBP12, and this complex then binds to and inhibits TOR function. Recent studies have shown that mTOR exist in two distinct protein complexes: mTOR complex 1 (mTORC1) and mTORC2. mTORC1 is sensitive to rapamycin, and contains mTOR, raptor (regulatory-associated protein of mTOR), and mLST8/G β L, whereas mTORC2 is rapamycin-insensitive and consists of mTOR, rictor, and mLST8/G β L (Sarbasov et al. 2005a; Wullschleger et al. 2006). In response to growth factor stimuli or nutritional status, mTORC1 controls mRNA translation via two mammalian proteins: eukaryotic initiation factor 4E (eIF-4E)-binding protein 1 (4E-BP1) and S6 kinase (S6K) (Hay and Sonenberg 2004). 4E-BP1 phosphorylation by mTOR releases eIF-4E from 4E-BP1, and free eIF-4E associates with eIF-4G and stimulates cap-dependent translation initiation (Hay and Sonenberg 2004). S6K was initially thought to up-regulate protein translation by enhancing translation of terminal oligopyrimidine tract (TOP) mRNAs, which encode ribosomal proteins and elongation factors, although recent studies do not support this notion (Wullschleger et al. 2006). Thus, the precise mechanisms by which S6K activation leads to increased translation are unclear at present.

The role of mTORC1-dependent translational control

in the development of cardiac hypertrophy was shown by studies using rapamycin. Rapamycin treatment attenuates agonist-induced hypertrophy in vitro (Sadoshima and Izumo 1995), and pressure overload hypertrophy in vivo (Shioi et al. 2003; McMullen et al. 2004a). Likewise, cardiac hypertrophy induced by Akt overexpression is effectively blocked by rapamycin treatment (Shioi et al. 2002; Shiojima et al. 2005), indicating that Akt-induced hypertrophy is predominantly mediated by the Akt-mTORC1 pathway. The mechanism by which Akt activates mTORC1 was elucidated by the identification of TSC2 as an Akt substrate. TSC2 forms a complex with TSC1 and negatively regulates mTOR through inhibition of Rheb (Ras homolog enriched in brain). Rheb is a Ras-like small GTPase that activates mTOR, and inactivated by the TSC1/2 complex that is a specific GTPase-activating protein (GAP) for Rheb. Phosphorylation of TSC2 by Akt inactivates the TSC1/2 complex, thereby releasing the negative regulation of TSC1/2 and activating mTOR (Hay and Sonenberg 2004). TSC2 is also phosphorylated by other kinases including AMP-activated protein kinase (AMPK). In contrast to Akt, phosphorylation of TSC2 by AMPK results in activation of the TSC1/2 complex, leading to mTORC1 down-regulation (Hay and Sonenberg 2004). Consistent with these findings, the adipose tissue-derived secreted factor adiponectin attenuates pathological cardiac growth by activating the AMPK pathway (Shibata et al. 2004), and direct pharmacological activation of AMPK in cardiac myocytes inhibits agonist-induced hypertrophy (Chan et al. 2004). Hypoxia was also shown to inhibit mTOR, and this inhibition is mediated by hypoxia-induced protein REDD1 acting downstream of Akt and upstream of TSC1/2 (Brugarolas et al. 2004). Thus, Akt signaling, AMPK signaling, and hypoxia signaling converge at TSC1/2 to modulate mTORC1 activity and cell growth. Further increasing the complexity of this regulatory system is the observation that Akt inhibits AMPK activity (Kovacic et al. 2003). The negative regulation of AMPK by Akt is thought to be mediated by phosphorylation of the AMPK $\alpha 1/\alpha 2$ subunit at Ser485/Ser491 by Akt and inhibition of subsequent Thr172 phosphorylation of AMPK α subunits by upstream AMPK kinases, or by the reduction of cellular AMP/ATP ratio by Akt (Hahn-Windgassen et al. 2005; Horman et al. 2006).

In contrast to mTORC1, mTORC2 is insensitive to rapamycin and regulates actin organization, probably through small G proteins Rho or Rac (Wulfschleger et al. 2006). Of note, mTORC2 was recently shown to phosphorylate Akt at S473 (Sarbasov et al. 2005b). Although Akt S473 kinase activity of mTORC2 has been shown to be activated by serum stimulation (Sarbasov et al. 2005b), whether mTORC2-dependent actin organization is mediated by Akt or how mTORC2 activity is regulated is largely unknown at present.

S6K (Fig. 3)

As mentioned earlier, S6K is activated by mTORC1, and has been implicated in body and organ size regulation.

S6K mutant flies show high incidence of lethality, and surviving flies display a decrease in body size due to a decrease in individual cell size (Montagne et al. 1999). There are two homologous S6Ks, S6K1 and S6K2, in mammals. Deletion of S6K1 results in mild growth retardation associated with up-regulation of S6K2 (Shima et al. 1998), whereas S6K2 deletion causes no obvious defect (Pende et al. 2004). Combined deletion of S6K1 and S6K2 results in reduced viability, and survivors exhibit similar body size to S6K1 single deletion (Pende et al. 2004). Importantly, translation of TOP mRNAs is still modulated by mitogens in a rapamycin-dependent manner in S6K1/S6K2-deficient cells. Likewise, TOP mRNA translation is also unaffected in mutant S6 protein knock-in mice in which all five phosphorylatable serine residues of S6 are substituted to alanines (Ruvinsky et al. 2005). These findings argue against the notion that TOP mRNA translation depends on S6 phosphorylation by S6K.

S6K activation is observed in IGF1R TG mice, active p110 α TG mice, PTEN KO mice and active Akt1 TG mice (Shioi et al. 2000, 2002; Crackower et al. 2002; McMullen et al. 2004b; Shiojima et al. 2005), whereas S6K down-regulation is detected in cardiac IR KO mice, dominant-negative p110 α TG mice, cardiac PDK1 KO mice, and Akt1 KO mice (Shioi et al. 2000; Shiojima et al. 2002; Mora et al. 2003; DeBosch et al. 2006a). Attenuation of cardiac hypertrophy by rapamycin is also associated with down-regulation of S6K (Shioi et al. 2003; McMullen et al. 2004a; Shiojima et al. 2005), and TG overexpression of wild-type or rapamycin-resistant S6K1 in the heart induces mild hypertrophy (a 10%–15% increase in heart size) with maintained contractility and no sign of heart pathology (McMullen et al. 2004c). These data suggest that S6K is an important mediator of hypertrophy downstream of the PI3K–Akt–mTORC1 pathway. On the other hand, deletion of S6K1 or S6K2 or both has no effect on exercise-induced or pressure overload-induced cardiac hypertrophy (McMullen et al. 2004c). Deletion of S6Ks also has no effect on heart size in IGF1R or active p110 α TG mice, and small heart phenotype of dominant-negative p110 α TG mice is not rescued by simultaneous overexpression of S6K1 in the heart (McMullen et al. 2004c). These findings suggest that S6K is not essential for the induction of hypertrophy in response to both physiological and pathological hypertrophic stimuli. Thus, hypertrophic effects of mTOR may be mediated by its downstream targets other than S6K (e.g., 4E-BP1) in the setting of functional S6K ablation.

FOXO (Fig. 3)

FOXO transcription factors belong to a large family of forkhead transcriptional regulators, which share a common feature in their DNA-binding forkhead domain (Barthel et al. 2005; Greer and Brunet 2005). The FOXO subfamily consists of four members: FOXO1/FKHR, FOXO3/FKHRL1, FOXO4/AFX and FOXO6. FOXO1, FOXO3, and FOXO4 are expressed in every tissue with

varying degrees, and FOXO1 is abundantly expressed in adipose tissues, FOXO3 in neuronal tissue, and FOXO4 in skeletal and cardiac muscles. FOXO6 is predominantly expressed in a specific region of the brain (Greer and Brunet 2005). The FOXO family of transcription factors is one of the major downstream targets of Akt. There are three Akt phosphorylation sites in FOXO1/3/4, which are conserved from worms to humans, although the third site is not conserved in FOXO6. Phosphorylation by Akt regulates subcellular localization of FOXO factors: Unphosphorylated FOXOs reside in the nucleus, and phosphorylation results in nuclear export of these factors. Thus, Akt negatively regulates transcriptional activity of FOXO proteins (Barthel et al. 2005; Greer and Brunet 2005). A similar mechanism has also been reported for the regulation of FOXA2/hepatocyte nuclear factor-3 β (HNF- β) transcriptional activity by Akt-dependent phosphorylation (Wolfrum et al. 2003). FOXO factors mainly function as transcriptional activators, although in some situations they also act as transcriptional repressors. A number of FOXO target genes have been identified, which regulate a variety of cellular functions including cell cycle arrest, DNA repair, cell death, differentiation, and glucose metabolism. In worms and flies, FOXO regulates life span downstream of the PI3K-Akt pathway. In mice, deletion of FOXO1 results in embryonic lethality at E10.5 due to defects in angiogenesis. FOXO3 KO mice exhibit age-dependent female infertility, and FOXO4 KO mice show no obvious phenotype (Greer and Brunet 2005). Thus, different FOXO factors appear to have distinct as well as overlapping functions in mice.

In cultured cardiac myocytes, the FOXO1/3/4 are phosphorylated in response to hypertrophic stimuli, and their nuclear localization is modulated by Akt-mediated phosphorylation. Phosphorylation of FOXO3 is increased in the heart in response to pressure overload, insulin injection, or Akt overexpression, and decreased in cardiac IR KO mice or Akt1 KO mice. Overexpression of FOXO3 inhibits growth factor or mechanical stretch-induced hypertrophy *in vitro* and reduces myocyte size *in vivo* (Skurk et al. 2005a). These findings indicate that FOXO factors are negative regulators of heart size, and that Akt-induced hypertrophy is partly mediated by inhibition of FOXO factors. The FOXO target genes that are thought to mediate its anti-hypertrophic effects include atrogen-1/MAFbx and MuRF1 E3 ubiquitin ligases (Sandri et al. 2004), which promote proteasome-mediated protein degradation (Hoffman and Nader 2004). Atrogen-1/MAFbx mRNA is down-regulated by Akt activation and up-regulated following Akt deactivation in the heart of inducible Akt1 TG mice (Skurk et al. 2005a), suggesting that Akt signaling simultaneously activates protein synthesis and inhibits protein degradation. This notion is consistent with the observation that, in inducible Akt TG mice, termination of Akt transgene expression after 2 wk of induction results in an extremely rapid decrease in heart size (almost complete reversal of ~80% increase in heart size within 72 h) (Shiojima et al. 2005). This rapid decrease in heart size is

presumably due to combinatorial effects of attenuated protein synthesis and enhanced protein degradation. Another possible mechanism by which FOXO factors negatively regulate heart size is through their ability to associate with and inhibit the transcriptional activity of myocardin (Pipes et al. 2006). Negative regulation of myocardin by FOXO4 has been shown in smooth muscle cells (Liu et al. 2005).

Akt signaling and coronary angiogenesis

Physiological cardiac hypertrophy is associated with normal or increased myocardial capillaries, whereas pathological hypertrophy is correlated with a reduction in capillary density (Hudlicka et al. 1992). For example, in human heart diseases such as aortic stenosis, dilated cardiomyopathy, and ischemic cardiomyopathy, there is a significant decrease in myocardial capillary density (Rakusan et al. 1992; Karch et al. 2005). Short-term Akt activation in inducible Akt1 TG mice induces physiological hypertrophy with maintained vascular density (Shiojima et al. 2005). Because there is an ~80% increase in heart size during short-term Akt activation, maintained vascular density in this situation indicates that coronary angiogenesis is enhanced to keep pace with the growth of the myocardium. VEGF and angiopoietin-2 (Ang-2) are key angiogenic growth factors induced by hypoxia (Pouyssegur et al. 2006), and expression of these two growth factors is enhanced by short-term Akt activation in the myocardium in a mTORC1-dependent manner (Shiojima et al. 2005). Ang-2 provides a destabilizing signal involved in initiating angiogenic sprouting in the presence of VEGF, and VEGF and Ang-2 are coinduced in or around the vessels undergoing active remodeling process (Yancopoulos et al. 2000). Furthermore, transgenic coexpression of VEGF and Ang-2 in the myocardium exhibits synergistic effects on induction of coronary angiogenesis (Visconti et al. 2002). Thus, Akt-mediated growth-promoting signals in cardiac muscle cells also act to enhance angiogenesis in the heart in a paracrine manner, providing a mechanism by which heart growth and angiogenesis is coordinately regulated. Similar observations have also been made in skeletal muscle cells: Akt activation in skeletal muscle cells results in myofiber growth associated with enhanced VEGF secretion and induces blood vessel recruitment (Takahashi et al. 2002). Because it has been shown that an increase in the level of HIF-1 protein is mediated by mTOR (Brugarolas et al. 2003), Akt-induced expression of VEGF and Ang-2 might be mediated by up-regulation of HIF-1 through mTOR activation.

Long-term Akt activation in the heart leads to pathological hypertrophy. Under these conditions, VEGF and Ang-2 are down-regulated, and capillary density is reduced. The causal role of impaired coronary angiogenesis on contractile dysfunction and heart failure was demonstrated by the observation that inhibition of coronary angiogenesis by decoy VEGF receptor during short-term Akt activation results in conversion from physiological to pathological hypertrophy and contractile dysfunction

(Shiojima et al. 2005). Likewise, in pressure overload-induced hypertrophy, VEGF blockade reduces capillary density and results in an accelerated transition from adaptive hypertrophy to heart failure (Izumiya et al. 2006). Thus, attenuated coronary angiogenesis in the setting of Akt-mediated cardiac growth plays a role in the development of cardiac pathology, and the balance between cardiac growth and coronary angiogenesis, rather than the extent of hypertrophy per se, is a critical determinant of physiological versus pathological hypertrophy (Fig. 4).

Decreased capillary density after prolonged Akt activation is associated with blunted S6K phosphorylation, suggesting that long-term Akt activation results in down-regulation of mTOR, leading to reduced expression of VEGF and Ang-2 and impaired coronary angiogenesis (Shiojima et al. 2005). However, the reason why short-term and long-term Akt activation exhibit differential effects on mTOR activity is unknown. In this regard, it has been shown that the hearts of conventional cardiac Akt1 TG mice are paradoxically more susceptible to ischemia/reperfusion injury, and this detrimental effect can be rescued by the expression of constitutively active p110 α subunit of PI3K. Furthermore, in these TG hearts, IR substrate-1 (IRS-1) expression and PI3K activity is reduced, suggesting the existence of PI3K-dependent but Akt-independent cardioprotective mechanisms (Nagoshi et al. 2005). One candidate molecule that could confer such cardioprotective actions is

Figure 4. Akt-mTOR-dependent myocyte growth and coronary angiogenesis. During Akt-mediated physiological cardiac growth, Akt-mTOR pathway is activated and promotes both myocyte growth and coronary angiogenesis, leading to maintained capillary density. However, prolonged Akt activation results in an imbalance between myocyte growth and coronary angiogenesis due to selective down-regulation of mTOR-mediated angiogenic responses, which leads to the development of pathological hypertrophy. Blockade of the VEGF signal in the early phase after Akt activation also results in impaired coronary angiogenesis and early transition from physiological to pathological hypertrophy. Thus, the relative balance between cardiac growth and coronary angiogenesis, rather than the extent of hypertrophy, is a critical determinant of physiological versus pathological cardiac hypertrophy.

SGK1, which is activated by PDK1 and positively regulates cardiomyocyte survival (Aoyama et al. 2005). Thus, it appears that an Akt-independent, PI3K-dependent signaling pathway is disrupted due to chronic Akt activation-induced IRS1 down-regulation, which might be mediated by Akt-dependent serine phosphorylation of IRS1 (O'Neill and Abel 2005). Similar negative feedback regulation of Akt signaling at the level of IR is also reported: Akt-dependent threonine phosphorylation of IR β subunit results in attenuation of insulin signaling (Morisco et al. 2005), and Akt-mediated inhibition of FOXO activity results in attenuated transcription of IR gene, which is a direct transcriptional target of FOXO factors (Puig and Tjian 2005). Taken together, one possible explanation for the differential effects of short-term versus long-term Akt activation on mTOR is that Akt-independent signals downstream of insulin/IGF receptors are required to maintain mTOR activity, and that such signals are disrupted by chronic Akt activation.

Impaired contractility in the setting of reduced capillary density suggests that contractile function is dependent on coronary angiogenesis. Notably, inhibition of angiogenesis attenuates Akt-mediated or pressure overload-induced cardiac growth (Shiojima et al. 2005; Izumiya et al. 2006), suggesting that the growth of the heart in response to both physiological and pathological stimuli is also dependent on coronary angiogenesis. It is well established that tumor growth is angiogenesis-dependent (Folkman 2001), and adipose tissue mass is regulated by the vasculature (Rupnick et al. 2002). Similarly, newly formed coronary vessels may promote cardiac growth by carrying nutrients and oxygen. Alternatively, the angiogenesis dependence of cardiac growth and function might be mediated by paracrine factors released from the vasculature (Folkman 1998). For example, it has been shown that normal development of liver or pancreas requires unknown paracrine factor(s) released from endothelial cells (Lammert et al. 2001; Matsumoto et al. 2001), and hepatocyte growth factor secreted from liver endothelial cells promotes hepatocyte proliferation (LeCouter et al. 2003). Since vessel formation during cardiac growth is dependent on paracrine signals from cardiac muscle cells (e.g., VEGF and angiopoietin-2), it is reasonable to hypothesize the existence of reciprocal cross-talk mechanisms between the vasculature and cardiac myocytes that coordinately regulate coronary angiogenesis, cardiac function, and overall heart size (Fig. 5). In this scenario, growth stimuli in cardiac muscle cells induce angiogenic growth factors, which recruit blood vessels. Blood vessels, on the other hand, secrete unidentified factors that promote cardiomyocyte growth and/or improve contractile function. Of note, treatment with a decoy VEGF receptor leads to a marked increase in VEGF expression from the myocardium, suggesting that VEGF exerts negative feedback signals to suppress VEGF expression in myocytes (Shiojima et al. 2005). Thus, it appears that complex mechanisms of cross-talk between cardiac myocytes and coronary vasculature orchestrate coronary angiogenesis, cardiac function, and heart growth.

Figure 5. Cross-talk between myocytes and endothelial cells during cardiac growth. During cardiac growth, myocytes secrete angiogenic growth factors such as VEGF and Ang-2 and promote coronary angiogenesis. On the other hand, endothelial cells may secrete a humoral factor(s) that promotes cardiac growth and/or maintains contractile function, because blockade of coronary angiogenesis results in attenuated heart growth and impaired contractility. Endothelial cells may also secrete a factor(s) in response to VEGF stimulation that inhibits VEGF expression in myocytes, because VEGF blockade results in a massive increase in VEGF expression levels in myocytes. Thus, complex cross-talk between cardiac myocytes and the vasculature regulates cardiac growth, coronary angiogenesis, and contractile function.

Akt signaling, cardiac protection, and contractile function

As discussed above, myocardial Akt signaling enhances coronary angiogenesis through the induction of angiogenic growth factors, and impaired angiogenesis in the presence of growth promoting stimuli plays a causal role in contractile dysfunction. Thus, enhanced coronary angiogenesis induced by Akt signaling may contribute to Akt-mediated improvement of cardiac function. However, it is likely that Akt signaling exerts beneficial actions on the heart through additional mechanisms.

Anti-apoptosis

Several lines of evidences support the notion that cardiomyocyte apoptosis plays a causal role in the development of heart failure, and that inhibition of cardiomyocyte apoptosis attenuates contractile dysfunction in heart failure (Foo et al. 2005). TG mice overexpressing IGF-I, an upstream effector of Akt signaling, display less myocyte apoptosis following myocardial infarction (Li et al. 1997). IGF-I administration also reduces myocardial apoptosis in response to ischemia/reperfusion injury in rats (Buerke et al. 1995), and IGF-1 functions as a survival factor for cultured cardiac myocytes exposed to the cardiotoxin doxorubicin (Wang et al. 1998). The cytoprotective effect of IGF-I on cultured cardiomyocytes can be abrogated by the PI3K inhibitor wortmannin or by the transduction of dominant-negative Akt1, whereas constitutively-active Akt1 protects cardiomyocytes from apoptosis in the absence of IGF-I (Matsui et al. 1999; Fujio et al. 2000). Thus, Akt signaling is both essential and sufficient for IGF-1 survival signals in cardiomyocytes in vitro. Furthermore, adenovirus-mediated Akt1 gene transfer in the heart diminishes cardiomyocyte

apoptosis and limits infarct size following ischemia/reperfusion injury (Fujio et al. 2000; Miao et al. 2000; Matsui et al. 2001), and ameliorate doxorubicin-induced contractile dysfunction (Taniyama and Walsh 2002). Thus, inhibition of cardiomyocyte apoptosis may be one of the mechanisms by which Akt signaling attenuates contractile dysfunction in the failing myocardium.

Regulation of Ca^{2+} cycling (Fig. 6)

Akt signaling may also improve contractile function by influencing myocardial Ca^{2+} cycling, which plays a critical role in contractility and relaxation of myocytes. Myocyte contraction is initially evoked by a small amount of Ca^{2+} influx through L-type Ca^{2+} channel (LTCC), which induces subsequent large-scale Ca^{2+} release from sarcoplasmic reticulum (SR) through ryanodine receptor (RyR) (Chien et al. 2003; Yano et al. 2005). Relaxation is mediated by reuptake of Ca^{2+} into SR through SR Ca^{2+} -ATPase (SERCA2), whose activity is regulated by phospholamban (PLB). This whole process of Ca^{2+} cycling is characterized by a transient increase in intracellular Ca^{2+} concentration (Ca^{2+} transient). Previous studies demonstrated that acute administration of IGF-I to both normal subjects and heart failure patients exerts positive

Figure 6. Regulation of Ca^{2+} cycling by Akt. In response to a small amount of Ca^{2+} influx through LTCC during membrane depolarization, a large-scale Ca^{2+} release from SR occurs through RyR, and induces muscle contraction. Reuptake of released Ca^{2+} into SR then occurs through SERCA2, which induces muscle relaxation. PLB is a negative regulator of SERCA2, and PLB phosphorylation results in down-regulation of PLB and increased SERCA2 activity. PKA positively regulates contraction through phosphorylation of at least four distinct substrates: LTCC, RyR, PLB, and I-1. I-1 phosphorylation by PKA results in activation of I-1, leading to down-regulation of PP1 activity and increased phosphorylation of PLB. PKC α , on the other hand, negatively regulates contraction through phosphorylation of I-1 at a different site from that phosphorylated by PKA. I-1 phosphorylation by PKC α results in inhibition of I-1, leading to up-regulation of PP1 activity and decreased phosphorylation of PLB. Akt appears to positively regulate contraction by increasing Ca^{2+} influx through LTCC (I_{CaL}), SERCA2 protein levels, and PLB phosphorylation levels (possibly through down-regulation of PP1). Whether these effects of Akt are mediated by direct phosphorylation of LTCC, SERCA2, or I-1 awaits further investigation.

inotropic effects and enhances contractile function (Donath et al. 1996, 1998). A positive inotropic effect of IGF-I is also observed in isolated cardiac myocytes, which is associated with increased peak Ca^{2+} transient and Ca^{2+} influx through LTCC (I_{CaL}) (Kinugawa et al. 1999; von Lewinski et al. 2003). Importantly, this positive inotropic effect of IGF-I on isolated myocytes is completely blocked by a PI3K inhibitor, and partially prevented by a LTCC blocker (von Lewinski et al. 2003). Furthermore, it was shown that inhibition of PI3K α or Akt blocks IGF-I- or PTEN deletion-induced increase in I_{CaL} (Sun et al. 2006). Increased I_{CaL} is also observed in myocytes isolated from Akt1 E40K TG hearts (Kim et al. 2003). Collectively, these data suggest that one possible explanation for Akt-mediated enhanced contractility is that Akt exerts positive inotropic effects by increasing I_{CaL} . How Akt regulates LTCC and I_{CaL} is unclear at present.

Several other mechanisms for Akt-mediated regulation of Ca^{2+} cycling are also proposed. It was shown that, in myocytes isolated from Akt1 E40K TG hearts, there is an increase in Ca^{2+} reuptake by SR associated with a marked increase in the amount of SERCA2 protein (Kim et al. 2003). SERCA2 regulates both relaxation and contractility, because decreased SERCA2 activity results in a reduction in Ca^{2+} store in SR, leading to decreased Ca^{2+} release through RyR and reduced contractility. Overexpression of SERCA2 enhances contractility in normal hearts and improves contractile dysfunction in heart failure models (del Monte and Hajjar 2003), and heterozygous deletion of SERCA2 gene results in a reduction in contractility and peak Ca^{2+} transient (Ji et al. 2000). Thus, Akt may exhibit positive inotropic effect by increasing the expression levels of SERCA2. The mechanisms by which Akt up-regulates SERCA2 protein levels are unknown.

Enhanced contractility and increased reuptake of Ca^{2+} is also reported in myocytes isolated from nuclear-targeted Akt1 TG hearts (Rota et al. 2005). Because there is no alteration in peak Ca transient, I_{CaL} , and SERCA2 protein levels in this case, increased contractility and Ca^{2+} reuptake is presumably due to enhanced SERCA2 activity. In fact, these myocytes exhibit increased phosphorylation of PLB associated with a decrease in protein phosphatase 1 (PP1) levels (Rota et al. 2005). PLB inhibits SERCA2 activity, and phosphorylation of PLB prevents its inhibitory effect on SERCA2 (Chien et al. 2003; Yano et al. 2005). PP1 dephosphorylates PLB and negatively regulates SERCA2 activity (Braz et al. 2004). Thus, another possible explanation of Akt-mediated enhanced contractility is that Akt enhances SERCA2 activity through down-regulation of PP1. PP1 is differentially regulated by protein kinase A (PKA) and PKC α (Champion and Kass 2004). PKA activates inhibitory-1 (I-1) protein, a negative regulator of PP1, and therefore up-regulates SERCA2 activity, whereas PKC α inhibits I-1 and negatively regulates SERCA2 (Braz et al. 2004). Whether down-regulation of PP1 by Akt is mediated by I-1 phosphorylation, as is the case with PKA, awaits further investigation.

Modulation of glucose/fatty acid metabolism (Fig. 7)

The heart is an energy-demanding organ, and myocardial substrate metabolism plays a key role in the maintenance of contractile function (Stanley et al. 2005). Under normal conditions, ATP is produced 10%–40% from oxidation of glucose and lactate and 60%–90% from β -oxidation of fatty acids. Fatty acids generate more ATP per gram of substrate than lactate or glucose, and are “energy efficient,” whereas glucose and lactate generate more ATP than fatty acids for each mole of oxygen and are “oxygen efficient” (Wolff et al. 2002). Therefore, if the supply of oxygen is limited, glucose oxidation will provide more energy per equal amount of oxygen and support more work than fatty acids. Consistent with this notion, heart function under ischemic condition is greater when the heart is oxidizing glucose than fatty acids (Stanley et al. 2005). Furthermore, there is a cross-regulatory mechanism between glucose/lactate oxidation and fatty acid oxidation, where glucose/lactate oxidation inhibits fatty acid oxidation and vice versa (Depre et al. 1999). Therefore, stimulation of glucose oxidation may be beneficial under ischemic conditions, and the cardioprotective effects of glucose–insulin–potassium (GIK) therapy or fatty acid oxidation inhibitors shown in clinical trials support this notion (Wolff et al. 2002).

Figure 7. Modulation of glucose/fatty acid metabolism by Akt. Glucose and fatty acids are two major sources of ATP synthesis in the heart. Oxidation of glucose or fatty acid generates the reducing equivalents (NADH and FADH_2), either directly or indirectly through the oxidation of acetyl-CoA in the TCA cycle. The reducing equivalents deliver electrons to the electron transport chain, leading to ATP synthesis through oxidative phosphorylation. Under normal conditions, 10%–40% of ATP is generated from glucose oxidation and 60%–90% from fatty acid oxidation. A shift of substrates from fatty acids to glucose leads to “oxygen-efficient” ATP synthesis and improves contractile function under ischemic condition. Akt may promote glucose oxidation by enhancing glucose uptake through glucose transporters and attenuate fatty acid oxidation through down-regulation of PPAR α and PGC-1, which transcriptionally activate the genes in fatty acid oxidation pathway. Thus, the positive inotropic effects of Akt may be in part mediated by promoting a shift of substrates for ATP synthesis from fatty acids to glucose.

Akt promotes glucose uptake by enhancing the membrane translocation of glucose transporter GLUT4 in part through phosphorylation of AS160 (Dugani and Klip 2005; Woodgett 2005). It was also shown that cardiac Akt overexpression results in down-regulation of peroxisome proliferator-activated receptor- α (PPAR α) and its coactivator PPAR γ coactivator-1 (PGC-1) (Cook et al. 2002), which transcriptionally activate the expression of molecules that regulate uptake and β -oxidation of fatty acids (Huss and Kelly 2005), suggesting that Akt may inhibit fatty acid oxidation through inhibition of PPAR α /PGC-1-dependent transcription and indirectly promote glucose/lactate oxidation. Thus, Akt signaling can enhance contractile function in the setting of ischemia preferentially by stimulating glucose oxidation. In fact, adenovirus-mediated overexpression of Akt in cultured cardiac myocytes results in enhanced glucose uptake and restoration of hypoxia-induced abnormalities in contractility and Ca²⁺ transient (Matsui et al. 2001).

Autocrine signals induced by Akt activation

It has been shown that transplantation of bone marrow-derived mesenchymal stem cells modified to overexpress Akt1 inhibit ventricular remodeling and improve contractile dysfunction after myocardial infarction (Mangi et al. 2003). Initially, this beneficial effect of Akt overexpression was thought to be due to enhanced myocardial regeneration. However, it was subsequently reported that improved ventricular function is observed as early as 72 h after cell transplantation, and conditioned media obtained from Akt-overexpressing mesenchymal stem cells also improve contractility, suggesting that paracrine factors released from mesenchymal stem cells in response to Akt activation account for the early improvement of contractile function (Gnecchi et al. 2005, 2006). Although the exact nature of paracrine factors induced by Akt is unknown, several growth factors including IGF-I are secreted from Akt-overexpressing mesenchymal stem cells (Gnecchi et al. 2006). By analogy, it is possible that factors secreted from cardiac myocytes in response to Akt activation may contribute to the Akt-mediated positive inotropic effect in an autocrine fashion.

Implications and future perspectives

In summary, recent studies suggest that Akt signaling in the heart coordinately regulates cardiac growth, contractile function, and coronary angiogenesis. These studies also provide potential insights about therapeutic strategies for heart diseases. First, because the imbalance between myocyte growth and coronary angiogenesis is a critical determinant of cardiac function and plays an important role in the transition from adaptive hypertrophy to heart failure, it may be advantageous to stimulate angiogenesis as part of a strategy to prevent or reverse heart failure in general (i.e., proangiogenic that is not restricted to ischemic cardiomyopathy). Likewise, it may

be possible to treat heart failure with a combination of anti-hypertrophic and proangiogenic agents, and the combined therapy might be more effective than each alone. Second, in clinical settings, agents that activate Akt signaling may not be suitable therapeutic targets for heart failure because sustained Akt activation is deleterious for the heart. Given that beneficial effects of Akt can be separable from its growth-promoting effect, downstream substrates of Akt that mediate its positive inotropic or cardioprotective effects could be promising therapeutic targets for heart failure. Thus, elucidation of downstream targets of Akt signaling in the heart will provide important clues to the development of novel therapeutic strategies for heart diseases.

References

- Ackah, E., Yu, J., Zoellner, S., Iwakiri, Y., Skurk, C., Shibata, R., Ouchi, N., Easton, R.M., Galasso, G., Birnbaum, M.J., et al. 2005. Akt1/protein kinase B α is critical for ischemic and VEGF-mediated angiogenesis. *J. Clin. Invest.* **115**: 2119–2127.
- Ackler, S., Ahmad, S., Tobias, C., Johnson, M.D., and Glazer, R.I. 2002. Delayed mammary gland involution in MMTV–AKT1 transgenic mice. *Oncogene* **21**: 198–206.
- Adini, I., Rabinovitz, I., Sun, J.F., Prendergast, G.C., and Benjamin, L.E. 2003. RhoB controls Akt trafficking and stage-specific survival of endothelial cells during vascular development. *Genes & Dev.* **17**: 2721–2732.
- Altomare, D.A. and Testa, J.R. 2005. Perturbations of the AKT signaling pathway in human cancer. *Oncogene* **24**: 7455–7464.
- An, D. and Rodrigues, B. 2006. The role of changes in cardiac metabolism in the development of diabetic cardiomyopathy. *Am. J. Physiol. Heart Circ. Physiol.* **291**: H1489–H1506.
- Andjelkovic, M., Jakubowicz, T., Cron, P., Ming, X.F., Han, J.W., and Hemmings, B.A. 1996. Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC-PK/PKB) promoted by serum and protein phosphatase inhibitors. *Proc. Natl. Acad. Sci.* **93**: 5699–5704.
- Antos, C.L., McKinsey, T.A., Frey, N., Kutschke, W., McAnally, J., Shelton, J.M., Richardson, J.A., Hill, J.A., and Olson, E.N. 2002. Activated glycogen synthase-3 β suppresses cardiac hypertrophy in vivo. *Proc. Natl. Acad. Sci.* **99**: 907–912.
- Aoyama, T., Matsui, T., Novikov, M., Park, J., Hemmings, B., and Rosenzweig, A. 2005. Serum and glucocorticoid-responsive kinase-1 regulates cardiomyocyte survival and hypertrophic response. *Circulation* **111**: 1652–1659.
- Badorff, C., Seeger, F.H., Zeiher, A.M., and Dimmeler, S. 2005. Glycogen synthase kinase 3 β inhibits myocardin-dependent transcription and hypertrophy induction through site-specific phosphorylation. *Circ. Res.* **97**: 645–654.
- Barthel, A., Schmoll, D., and Unterman, T.G. 2005. FoxO proteins in insulin action and metabolism. *Trends Endocrinol. Metab.* **16**: 183–189.
- Belke, D.D., Betuing, S., Tuttle, M.J., Graveleau, C., Young, M.E., Pham, M., Zhang, D., Cooksey, R.C., McClain, D.A., Litwin, S.E., et al. 2002. Insulin signaling coordinately regulates cardiac size, metabolism, and contractile protein isoform expression. *J. Clin. Invest.* **109**: 629–639.
- Bellacosa, A., Chan, T.O., Ahmed, N.N., Datta, K., Malstrom, S., Stokoe, D., McCormick, F., Feng, J., and Tsichlis, P. 1998. Akt activation by growth factors is a multiple-step process: The role of the PH domain. *Oncogene* **17**: 313–325.

- Bernal-Mizrachi, E., Wen, W., Stahlhut, S., Welling, C.M., and Permutt, M.A. 2001. Islet β cell expression of constitutively active Akt1/PKB α induces striking hypertrophy, hyperplasia, and hyperinsulinemia. *J. Clin. Invest.* **108**: 1631–1638.
- Braunwald, E. and Bristow, M.R. 2000. Congestive heart failure: Fifty years of progress. *Circulation* **102** (Suppl. 4): IV14–IV23.
- Braz, J.C., Gregory, K., Pathak, A., Zhao, W., Sahin, B., Klevitsky, R., Kimball, T.F., Lorenz, J.N., Nairn, A.C., Liggett, S.B., et al. 2004. PKC- α regulates cardiac contractility and propensity toward heart failure. *Nat. Med.* **10**: 248–254.
- Brazil, D.P., Park, J., and Hemmings, B.A. 2002. PKB binding proteins. Getting in on the Akt. *Cell* **111**: 293–303.
- Brazil, D.P., Yang, Z.Z., and Hemmings, B.A. 2004. Advances in protein kinase B signalling: AKTion on multiple fronts. *Trends Biochem. Sci.* **29**: 233–242.
- Brugarolas, J.B., Vazquez, F., Reddy, A., Sellers, W.R., and Kaelin Jr., W.G. 2003. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell* **4**: 147–158.
- Brugarolas, J., Lei, K., Hurley, R.L., Manning, B.D., Reiling, J.H., Hafen, E., Witters, L.A., Ellisen, L.W., and Kaelin Jr., W.G. 2004. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes & Dev.* **18**: 2893–2904.
- Buckingham, M., Meilhac, S., and Zaffran, S. 2005. Building the mammalian heart from two sources of myocardial cells. *Nat. Rev. Genet.* **6**: 826–835.
- Buerke, M., Murohara, T., Skurk, C., Nuss, C., Tomaselli, K., and Lefer, A.M. 1995. Cardioprotective effect of insulin-like growth factor I in myocardial ischemia followed by reperfusion. *Proc. Natl. Acad. Sci.* **92**: 8031–8035.
- Camper-Kirby, D., Welch, S., Walker, A., Shiraishi, I., Setchell, K.D., Schaefer, E., Kajstura, J., Anversa, P., and Sussman, M.A. 2001. Myocardial Akt activation and gender: Increased nuclear activity in females versus males. *Circ. Res.* **88**: 1020–1027.
- Champion, H.C. and Kass, D.A. 2004. Calcium handler mishandles heart. *Nat. Med.* **10**: 239–240.
- Chan, A.Y., Soltys, C.L., Young, M.E., Proud, C.G., and Dyck, J.R. 2004. Activation of AMP-activated protein kinase inhibits protein synthesis associated with hypertrophy in the cardiac myocyte. *J. Biol. Chem.* **279**: 32771–32779.
- Chen, W.S., Xu, P.Z., Gottlob, K., Chen, M.L., Sokol, K., Shiyanova, T., Roninson, I., Weng, W., Suzuki, R., Tobe, K., et al. 2001. Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. *Genes & Dev.* **15**: 2203–2208.
- Chen, H., Shi, S., Acosta, L., Li, W., Lu, J., Bao, S., Chen, Z., Yang, Z., Schneider, M.D., Chien, K.R., et al. 2004. BMP10 is essential for maintaining cardiac growth during murine cardiogenesis. *Development* **131**: 2219–2231.
- Chen, J., Somanath, P.R., Razorenova, O., Chen, W.S., Hay, N., Bornstein, P., and Byzova, T.V. 2005. Akt1 regulates pathological angiogenesis, vascular maturation and permeability in vivo. *Nat. Med.* **11**: 1188–1196.
- Chien, K.R., Ross Jr., J., and Hoshijima, M. 2003. Calcium and heart failure: The cycle game. *Nat. Med.* **9**: 508–509.
- Cho, H., Mu, J., Kim, J.K., Thorvaldsen, J.L., Chu, Q., Crenshaw III, E.B., Kaestner, K.H., Bartolomei, M.S., Shulman, G.I., and Birnbaum, M.J. 2001a. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB β). *Science* **292**: 1728–1731.
- Cho, H., Thorvaldsen, J.L., Chu, Q., Feng, F., and Birnbaum, M.J. 2001b. Akt1/pkb α is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J. Biol. Chem.* **276**: 38349–38352.
- Cho, K.S., Lee, J.H., Kim, S., Kim, D., Koh, H., Lee, J., Kim, C., Kim, J., and Chung, J. 2001. Drosophila phosphoinositide-dependent kinase-1 regulates apoptosis and growth via the phosphoinositide 3-kinase-dependent signaling pathway. *Proc. Natl. Acad. Sci.* **98**: 6144–6149.
- Condorelli, G., Drusco, A., Stassi, G., Bellacosa, A., Roncarati, R., Iaccarino, G., Russo, M.A., Gu, Y., Dalton, N., Chung, C., et al. 2002. Akt induces enhanced myocardial contractility and cell size in vivo in transgenic mice. *Proc. Natl. Acad. Sci.* **99**: 12333–12338.
- Cook, S.A., Matsui, T., Li, L., and Rosenzweig, A. 2002. Transcriptional effects of chronic Akt activation in the heart. *J. Biol. Chem.* **277**: 22528–22533.
- Crackower, M.A., Oudit, G.Y., Kozieradzki, I., Sarao, R., Sun, H., Sasaki, T., Hirsch, E., Suzuki, A., Shioi, T., Irie-Sasaki, J., et al. 2002. Regulation of myocardial contractility and cell size by distinct PI3K–PTEN signaling pathways. *Cell* **110**: 737–749.
- Crisostomo, L.L., Araujo, L.M., Camara, E., Carvalho, C., Silva, F.A., Vieira, M., and Rabelo Jr., A. 1999. Comparison of left ventricular mass and function in obese versus nonobese women <40 years of age. *Am. J. Cardiol.* **84**: 1127–1129.
- Datta, S.R., Brunet, A., and Greenberg, M.E. 1999. Cellular survival: A play in three Akts. *Genes & Dev.* **13**: 2905–2927.
- DeBosch, B., Treskov, I., Lupu, T.S., Weinheimer, C., Kovacs, A., Courtois, M., and Muslin, A.J. 2006a. Akt1 is required for physiological cardiac growth. *Circulation* **113**: 2097–2104.
- DeBosch, B.J., Sambandam, N., Weinheimer, C.S., Courtois, M., and Muslin, A.J. 2006b. AKT2 regulates cardiac metabolism and cardiomyocyte survival. *J. Biol. Chem.* **281**: 32841–32851.
- Delaughter, M.C., Taffet, G.E., Fiorotto, M.L., Entman, M.L., and Schwartz, R.J. 1999. Local insulin-like growth factor I expression induces physiologic, then pathologic, cardiac hypertrophy in transgenic mice. *FASEB J.* **13**: 1923–1929.
- del Monte, F. and Hajjar, R.J. 2003. Targeting calcium cycling proteins in heart failure through gene transfer. *J. Physiol.* **546**: 49–61.
- Depre, C., Vanoverschelde, J.L., and Taegtmeyer, H. 1999. Glucose for the heart. *Circulation* **99**: 578–588.
- de Simone, G., Scalfi, L., Galderisi, M., Celentano, A., Di Biase, G., Tammaro, P., Garofalo, M., Mureddu, G.F., de Divitiis, O., and Contaldo, F. 1994. Cardiac abnormalities in young women with anorexia nervosa. *Br. Heart J.* **71**: 287–292.
- Doble, B.W. and Woodgett, J.R. 2003. GSK-3: Tricks of the trade for a multi-tasking kinase. *J. Cell Sci.* **116**: 1175–1186.
- Donath, M.Y., Jenni, R., Brunner, H.P., Anrig, M., Kohli, S., Glatz, Y., and Froesch, E.R. 1996. Cardiovascular and metabolic effects of insulin-like growth factor I at rest and during exercise in humans. *J. Clin. Endocrinol. Metab.* **81**: 4089–4094.
- Donath, M.Y., Sutsch, G., Yan, X.W., Piva, B., Brunner, H.P., Glatz, Y., Zapf, J., Follath, F., Froesch, E.R., and Kiowski, W. 1998. Acute cardiovascular effects of insulin-like growth factor I in patients with chronic heart failure. *J. Clin. Endocrinol. Metab.* **83**: 3177–3183.
- Dong, L.Q. and Liu, F. 2005. PDK2: The missing piece in the receptor tyrosine kinase signaling pathway puzzle. *Am. J. Physiol. Endocrinol. Metab.* **289**: E187–E196.
- Du, K. and Tsichlis, P.N. 2005. Regulation of the Akt kinase by interacting proteins. *Oncogene* **24**: 7401–7409.
- Dugani, C.B. and Klip, A. 2005. Glucose transporter 4: Cycling, compartments and controversies. *EMBO Rep.* **6**: 1137–1142.
- Easton, R.M., Cho, H., Roovers, K., Shineman, D.W., Mizrahi, M., Forman, M.S., Lee, V.M., Szabolcs, M., de Jong, R., Oltersdorf, T., et al. 2005. Role for Akt3/protein kinase B γ in attainment of normal brain size. *Mol. Cell. Biol.* **25**: 1869–

- 1878.
- Efstratiadis, A. 1998. Genetics of mouse growth. *Int. J. Dev. Biol.* **42**: 955–976.
- Folkman, J. 1998. Is tissue mass regulated by vascular endothelial cells? Prostate as the first evidence. *Endocrinology* **139**: 441–442.
- Folkman, J. 2001. Angiogenesis. In *Harrison's principles of internal medicine* (eds. E. Braunwald et al.), pp. 517–530. McGraw-Hill, New York.
- Foo, R.S., Mani, K., and Kitsis, R.N. 2005. Death begets failure in the heart. *J. Clin. Invest.* **115**: 565–571.
- Frey, N. and Olson, E.N. 2003. Cardiac hypertrophy: The good, the bad, and the ugly. *Annu. Rev. Physiol.* **65**: 45–79.
- Fujio, Y., Nguyen, T., Wencker, D., Kitsis, R.N., and Walsh, K. 2000. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. *Circulation* **101**: 660–667.
- Fukumoto, S., Hsieh, C.M., Maemura, K., Layne, M.D., Yet, S.F., Lee, K.H., Matsui, T., Rosenzweig, A., Taylor, W.G., Rubin, J.S., et al. 2001. Akt participation in the Wnt signaling pathway through Dishevelled. *J. Biol. Chem.* **276**: 17479–17483.
- Gao, T., Furnari, F., and Newton, A.C. 2005. PHLPP: A phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. *Mol. Cell* **18**: 13–24.
- Garofalo, R.S., Orena, S.J., Rafidi, K., Torchia, A.J., Stock, J.L., Hildebrandt, A.L., Coskran, T., Black, S.C., Brees, D.J., Wicks, J.R., et al. 2003. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB β . *J. Clin. Invest.* **112**: 197–208.
- Garratt, A.N., Ozcelik, C., and Birchmeier, C. 2003. ErbB2 pathways in heart and neural diseases. *Trends Cardiovasc. Med.* **13**: 80–86.
- George, S., Rochford, J.J., Wolfrum, C., Gray, S.L., Schinner, S., Wilson, J.C., Soos, M.A., Murgatroyd, P.R., Williams, R.M., Acerini, C.L., et al. 2004. A family with severe insulin resistance and diabetes due to a mutation in AKT2. *Science* **304**: 1325–1328.
- Gerdes, A.M., Kellerman, S.E., Moore, J.A., Muffly, K.E., Clark, L.C., Reaves, P.Y., Malec, K.B., McKeown, P.P., and Schocken, D.D. 1992. Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. *Circulation* **86**: 426–430.
- Ghosh-Choudhury, N., Abboud, S.L., Nishimura, R., Celeste, A., Mahimainathan, L., and Choudhury, G.G. 2002. Requirement of BMP-2-induced phosphatidylinositol 3-kinase and Akt serine/threonine kinase in osteoblast differentiation and Smad-dependent BMP-2 gene transcription. *J. Biol. Chem.* **277**: 33361–33368.
- Gnecchi, M., He, H., Liang, O.D., Melo, L.G., Morello, F., Mu, H., Noiseux, N., Zhang, L., Pratt, R.E., Ingwall, J.S., et al. 2005. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat. Med.* **11**: 367–368.
- Gnecchi, M., He, H., Noiseux, N., Liang, O.D., Zhang, L., Morello, F., Mu, H., Melo, L.G., Pratt, R.E., Ingwall, J.S., et al. 2006. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J.* **20**: 661–669.
- Greer, E.L. and Brunet, A. 2005. FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene* **24**: 7410–7425.
- Hahn-Windgassen, A., Nogueira, V., Chen, C.C., Skeen, J.E., Sonenberg, N., and Hay, N. 2005. Akt activates the mammalian target of rapamycin by regulating cellular ATP level and AMPK activity. *J. Biol. Chem.* **280**: 32081–32089.
- Haq, S., Choukroun, G., Kang, Z.B., Ranu, H., Matsui, T., Rosenzweig, A., Molkentin, J.D., Alessandrini, A., Woodgett, J., Hajjar, R., et al. 2000. Glycogen synthase kinase-3 β is a negative regulator of cardiomyocyte hypertrophy. *J. Cell Biol.* **151**: 117–130.
- Haq, S., Michael, A., Andreucci, M., Bhattacharya, K., Dotto, P., Walters, B., Woodgett, J., Kilter, H., and Force, T. 2003. Stabilization of β -catenin by a Wnt-independent mechanism regulates cardiomyocyte growth. *Proc. Natl. Acad. Sci.* **100**: 4610–4615.
- Hardt, S.E., Tomita, H., Katus, H.A., and Sadoshima, J. 2004. Phosphorylation of eukaryotic translation initiation factor 2B ϵ by glycogen synthase kinase-3 β regulates β -adrenergic cardiac myocyte hypertrophy. *Circ. Res.* **94**: 926–935.
- Hay, N. and Sonenberg, N. 2004. Upstream and downstream of mTOR. *Genes & Dev.* **18**: 1926–1945.
- Heineke, J. and Molkentin, J.D. 2006. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat. Rev. Mol. Cell Biol.* **7**: 589–600.
- Hoffman, E.P. and Nader, G.A. 2004. Balancing muscle hypertrophy and atrophy. *Nat. Med.* **10**: 584–585.
- Horman, S., Vertommen, D., Heath, R., Neumann, D., Mouton, V., Woods, A., Schlattner, U., Wallimann, T., Carling, D., Hue, L., et al. 2006. Insulin antagonizes ischemia-induced Thr172 phosphorylation of AMP-activated protein kinase α -subunits in heart via hierarchical phosphorylation of Ser485/491. *J. Biol. Chem.* **281**: 5335–5340.
- Hu, P., Zhang, D., Swenson, L., Chakrabarti, G., Abel, E.D., and Litwin, S.E. 2003. Minimally invasive aortic banding in mice: Effects of altered cardiomyocyte insulin signaling during pressure overload. *Am. J. Physiol. Heart Circ. Physiol.* **285**: H1261–H1269.
- Hudlicka, O. and Brown, M.D. 1996. Postnatal growth of the heart and its blood vessels. *J. Vasc. Res.* **33**: 266–287.
- Hudlicka, O., Brown, M., and Egginton, S. 1992. Angiogenesis in skeletal and cardiac muscle. *Physiol. Rev.* **72**: 369–417.
- Hue, L., Beauloye, C., Marsin, A.S., Bertrand, L., Horman, S., and Rider, M.H. 2002. Insulin and ischemia stimulate glycolysis by acting on the same targets through different and opposing signaling pathways. *J. Mol. Cell. Cardiol.* **34**: 1091–1097.
- Huss, J.M. and Kelly, D.P. 2005. Mitochondrial energy metabolism in heart failure: A question of balance. *J. Clin. Invest.* **115**: 547–555.
- Hutchinson, J., Jin, J., Cardiff, R.D., Woodgett, J.R., and Muller, W.J. 2001. Activation of Akt (protein kinase B) in mammary epithelium provides a critical cell survival signal required for tumor progression. *Mol. Cell. Biol.* **21**: 2203–2212.
- Izumiya, Y., Shiojima, I., Sato, K., Sawyer, D.B., Colucci, W.S., and Walsh, K. 2006. Vascular endothelial growth factor blockade promotes the transition from compensatory cardiac hypertrophy to failure in response to pressure overload. *Hypertension* **47**: 887–893.
- Ji, Y., Lalli, M.J., Babu, G.J., Xu, Y., Kirkpatrick, D.L., Liu, L.H., Chiamvimonvat, N., Walsh, R.A., Shull, G.E., and Periasamy, M. 2000. Disruption of a single copy of the SERCA2 gene results in altered Ca²⁺ homeostasis and cardiomyocyte function. *J. Biol. Chem.* **275**: 38073–38080.
- Karch, R., Neumann, F., Ullrich, R., Neumuller, J., Podesser, B.K., Neumann, M., and Schreiner, W. 2005. The spatial pattern of coronary capillaries in patients with dilated, ischemic, or inflammatory cardiomyopathy. *Cardiovasc. Pathol.* **14**: 135–144.
- Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L. 1950. *The biology of human starvation*. University of Minnesota Press, Minneapolis, MN.

- Kim, Y.K., Kim, S.J., Yatani, A., Huang, Y., Castelli, G., Vatner, D.E., Liu, J., Zhang, Q., Diaz, G., Zieba, R., et al. 2003. Mechanism of enhanced cardiac function in mice with hypertrophy induced by overexpressed Akt. *J. Biol. Chem.* **278**: 47622–47628.
- Kinugawa, S., Tsutsui, H., Ide, T., Nakamura, R., Arimura, K., Egashira, K., and Takeshita, A. 1999. Positive inotropic effect of insulin-like growth factor-1 on normal and failing cardiac myocytes. *Cardiovasc. Res.* **43**: 157–164.
- Klinz, F., Bloch, W., Addicks, K., and Hescheler, J. 1999. Inhibition of phosphatidylinositol-3-kinase blocks development of functional embryonic cardiomyocytes. *Exp. Cell Res.* **247**: 79–83.
- Kovacic, S., Soltys, C.L., Barr, A.J., Shiojima, I., Walsh, K., and Dyck, J.R. 2003. Akt activity negatively regulates phosphorylation of AMP-activated protein kinase in the heart. *J. Biol. Chem.* **278**: 39422–39427.
- Kozma, S.C. and Thomas, G. 2002. Regulation of cell size in growth, development and human disease: PI3K, PKB and S6K. *Bioessays* **24**: 65–71.
- Lammert, E., Cleaver, O., and Melton, D. 2001. Induction of pancreatic differentiation by signals from blood vessels. *Science* **294**: 564–567.
- Lawlor, M.A., Mora, A., Ashby, P.R., Williams, M.R., Murray-Tait, V., Malone, L., Prescott, A.R., Lucocq, J.M., and Alessi, D.R. 2002. Essential role of PDK1 in regulating cell size and development in mice. *EMBO J.* **21**: 3728–3738.
- LeCouter, J., Moritz, D.R., Li, B., Phillips, G.L., Liang, X.H., Gerber, H.P., Hillan, K.J., and Ferrara, N. 2003. Angiogenesis-independent endothelial protection of liver: Role of VEGFR-1. *Science* **299**: 890–893.
- Levy, D., Garrison, R.J., Savage, D.D., Kannel, W.B., and Castelli, W.P. 1990. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N. Engl. J. Med.* **322**: 1561–1566.
- Li, Q., Li, B., Wang, X., Leri, A., Jana, K.P., Liu, Y., Kajstura, J., Baserga, R., and Anversa, P. 1997. Overexpression of insulin-like growth factor-1 in mice protects from myocyte death after infarction, attenuating ventricular dilation, wall stress, and cardiac hypertrophy. *J. Clin. Invest.* **100**: 1991–1999.
- Liu, Z.P., Wang, Z., Yanagisawa, H., and Olson, E.N. 2005. Phenotypic modulation of smooth muscle cells through interaction of Foxo4 and myocardin. *Dev. Cell* **9**: 261–270.
- Luo, J., McMullen, J.R., Sobkiw, C.L., Zhang, L., Dorfman, A.L., Sherwood, M.C., Logsdon, M.N., Horner, J.W., DePinho, R.A., Izumo, S., et al. 2005. Class IA phosphoinositide 3-kinase regulates heart size and physiological cardiac hypertrophy. *Mol. Cell. Biol.* **25**: 9491–9502.
- Majumder, P.K., Yeh, J.J., George, D.J., Febbo, P.G., Kum, J., Xue, Q., Bikoff, R., Ma, H., Kantoff, P.W., Golub, T.R., et al. 2003. Prostate intraepithelial neoplasia induced by prostate restricted Akt activation: The MPAKT model. *Proc. Natl. Acad. Sci.* **100**: 7841–7846.
- Malstrom, S., Tili, E., Kappes, D., Ceci, J.D., and Tsichlis, P.N. 2001. Tumor induction by an Lck-MyrAkt transgene is delayed by mechanisms controlling the size of the thymus. *Proc. Natl. Acad. Sci.* **98**: 14967–14972.
- Mangi, A.A., Noisieux, N., Kong, D., He, H., Rezvani, M., Ingwall, J.S., and Dzau, V.J. 2003. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat. Med.* **9**: 1195–1201.
- Marvin, M.J., Di Rocco, G., Gardiner, A., Bush, S.M., and Lassar, A.B. 2001. Inhibition of Wnt activity induces heart formation from posterior mesoderm. *Genes & Dev.* **15**: 316–327.
- Matsui, T., Li, L., del Monte, F., Fukui, Y., Franke, T.F., Hajjar, R.J., and Rosenzweig, A. 1999. Adenoviral gene transfer of activated phosphatidylinositol 3'-kinase and Akt inhibits apoptosis of hypoxic cardiomyocytes in vitro. *Circulation* **100**: 2373–2379.
- Matsui, T., Tao, J., del Monte, F., Lee, K.H., Li, L., Picard, M., Force, T.L., Franke, T.F., Hajjar, R.J., and Rosenzweig, A. 2001. Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo. *Circulation* **104**: 330–335.
- Matsui, T., Li, L., Wu, J.C., Cook, S.A., Nagoshi, T., Picard, M.H., Liao, R., and Rosenzweig, A. 2002. Phenotypic spectrum caused by transgenic overexpression of activated Akt in the heart. *J. Biol. Chem.* **277**: 22896–22901.
- Matsumoto, K., Yoshitomi, H., Rossant, J., and Zaret, K.S. 2001. Liver organogenesis promoted by endothelial cells prior to vascular function. *Science* **294**: 559–563.
- McDevitt, T.C., Laflamme, M.A., and Murry, C.E. 2005. Proliferation of cardiomyocytes derived from human embryonic stem cells is mediated via the IGF/PI 3-kinase/Akt signaling pathway. *J. Mol. Cell. Cardiol.* **39**: 865–873.
- McMullen, J.R., Shioi, T., Zhang, L., Tarnavski, O., Sherwood, M.C., Kang, P.M., and Izumo, S. 2003. Phosphoinositide 3-kinase(p110 α) plays a critical role for the induction of physiological, but not pathological, cardiac hypertrophy. *Proc. Natl. Acad. Sci.* **100**: 12355–12360.
- McMullen, J.R., Sherwood, M.C., Tarnavski, O., Zhang, L., Dorfman, A.L., Shioi, T., and Izumo, S. 2004a. Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation* **109**: 3050–3055.
- McMullen, J.R., Shioi, T., Huang, W.Y., Zhang, L., Tarnavski, O., Bisping, E., Schinke, M., Kong, S., Sherwood, M.C., Brown, J., et al. 2004b. The insulin-like growth factor 1 receptor induces physiological heart growth via the phosphoinositide 3-kinase(p110 α) pathway. *J. Biol. Chem.* **279**: 4782–4793.
- McMullen, J.R., Shioi, T., Zhang, L., Tarnavski, O., Sherwood, M.C., Dorfman, A.L., Longnus, S., Pende, M., Martin, K.A., Blenis, J., et al. 2004c. Deletion of ribosomal S6 kinases does not attenuate pathological, physiological, or insulin-like growth factor 1 receptor-phosphoinositide 3-kinase-induced cardiac hypertrophy. *Mol. Cell. Biol.* **24**: 6231–6240.
- Miao, W., Luo, Z., Kitsis, R.N., and Walsh, K. 2000. Intracoronary, adenovirus-mediated Akt gene transfer in heart limits infarct size following ischemia-reperfusion injury in vivo. *J. Mol. Cell. Cardiol.* **32**: 2397–2402.
- Michael, A., Haq, S., Chen, X., Hsich, E., Cui, L., Walters, B., Shao, Z., Bhattacharya, K., Kilter, H., Huggins, G., et al. 2004. Glycogen synthase kinase-3 β regulates growth, calcium homeostasis, and diastolic function in the heart. *J. Biol. Chem.* **279**: 21383–21393.
- Molkentin, J. and Dorn, I.G. 2001. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Annu. Rev. Physiol.* **63**: 391–426.
- Montagne, J., Stewart, M.J., Stocker, H., Hafen, E., Kozma, S.C., and Thomas, G. 1999. *Drosophila* S6 kinase: A regulator of cell size. *Science* **285**: 2126–2129.
- Moon, R.T., Kohn, A.D., De Ferrari, G.V., and Kaykas, A. 2004. WNT and β -catenin signalling: Diseases and therapies. *Nat. Rev. Genet.* **5**: 691–701.
- Mora, A., Davies, A.M., Bertrand, L., Sharif, I., Budas, G.R., Jovanovic, S., Mouton, V., Kahn, C.R., Lucocq, J.M., Gray, G.A., et al. 2003. Deficiency of PDK1 in cardiac muscle results in heart failure and increased sensitivity to hypoxia. *EMBO J.* **22**: 4666–4676.
- Mora, A., Komander, D., van Aalten, D.M., and Alessi, D.R. 2004. PDK1, the master regulator of AGC kinase signal

- transduction. *Semin. Cell Dev. Biol.* **15**: 161–170.
- Morisco, C., Seta, K., Hardt, S.E., Lee, Y., Vatner, S.F., and Sadoshima, J. 2001. Glycogen synthase kinase 3 β regulates GATA4 in cardiac myocytes. *J. Biol. Chem.* **276**: 28586–28597.
- Morisco, C., Condorelli, G., Trimarco, V., Bellis, A., Marrone, C., Condorelli, G., Sadoshima, J., and Trimarco, B. 2005. Akt mediates the cross-talk between β -adrenergic and insulin receptors in neonatal cardiomyocytes. *Circ. Res.* **96**: 180–188.
- Mukai, Y., Rikitake, Y., Shiojima, I., Wolfrum, S., Satoh, M., Takeshita, K., Hiroi, Y., Salomone, S., Kim, H.H., Benjamin, L.E., et al. 2006. Decreased vascular lesion formation in mice with inducible endothelial-specific expression of protein kinase Akt. *J. Clin. Invest.* **116**: 334–343.
- Naga Prasad, S.V., Esposito, G., Mao, L., Koch, W.J., and Rockman, H.A. 2000. G β -dependent phosphoinositide 3-kinase activation in hearts with in vivo pressure overload hypertrophy. *J. Biol. Chem.* **275**: 4693–4698.
- Nagoshi, T., Matsui, T., Aoyama, T., Leri, A., Anversa, P., Li, L., Ogawa, W., del Monte, F., Gwathmey, J.K., Grazette, L., et al. 2005. PI3K rescues the detrimental effects of chronic Akt activation in the heart during ischemia/reperfusion injury. *J. Clin. Invest.* **115**: 2128–2138.
- Naito, A.T., Tominaga, A., Oyamada, M., Oyamada, Y., Shiraiishi, I., Monzen, K., Komuro, I., and Takamatsu, T. 2003. Early stage-specific inhibitions of cardiomyocyte differentiation and expression of Csx/Nkx-2.5 and GATA-4 by phosphatidylinositol 3-kinase inhibitor LY294002. *Exp. Cell Res.* **291**: 56–69.
- Naito, A.T., Akazawa, H., Takano, H., Minamino, T., Nagai, T., Aburatani, H., and Komuro, I. 2005. Phosphatidylinositol 3-kinase-Akt pathway plays a critical role in early cardiomyogenesis by regulating canonical Wnt signaling. *Circ. Res.* **97**: 144–151.
- Nakamura, T., Sano, M., Songyang, Z., and Schneider, M.D. 2003. A Wnt- and β -catenin-dependent pathway for mammalian cardiac myogenesis. *Proc. Natl. Acad. Sci.* **100**: 5834–5839.
- Neri Serneri, G.G., Boddi, M., Modesti, P.A., Cecioni, I., Coppo, M., Padeletti, L., Michelucci, A., Colella, A., and Galanti, G. 2001. Increased cardiac sympathetic activity and insulin-like growth factor-I formation are associated with physiological hypertrophy in athletes. *Circ. Res.* **89**: 977–982.
- Olson, E.N. and Schneider, M.D. 2003. Sizing up the heart: Development redux in disease. *Genes & Dev.* **17**: 1937–1956.
- O'Neill, B.T. and Abel, E.D. 2005. Akt1 in the cardiovascular system: Friend or foe? *J. Clin. Invest.* **115**: 2059–2064.
- Oudit, G.Y., Sun, H., Kerfant, B.G., Crackower, M.A., Penninger, J.M., and Backx, P.H. 2004. The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. *J. Mol. Cell. Cardiol.* **37**: 449–471.
- Pasumarthi, K.B. and Field, L.J. 2002. Cardiomyocyte cell cycle regulation. *Circ. Res.* **90**: 1044–1054.
- Patrucco, E., Notte, A., Barberis, L., Selvetella, G., Maffei, A., Brancaccio, M., Marengo, S., Russo, G., Azzolino, O., Rybalkin, S.D., et al. 2004. PI3K γ modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. *Cell* **118**: 375–387.
- Pende, M., Um, S.H., Mieulet, V., Sticker, M., Goss, V.L., Mestian, J., Mueller, M., Fumagalli, S., Kozma, S.C., and Thomas, G. 2004. S6K1 $^{-/-}$ /S6K2 $^{-/-}$ mice exhibit perinatal lethality and rapamycin-sensitive 5'-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. *Mol. Cell. Biol.* **24**: 3112–3124.
- Peng, X.D., Xu, P.Z., Chen, M.L., Hahn-Windgassen, A., Skeen, J., Jacobs, J., Sundararajan, D., Chen, W.S., Crawford, S.E., Coleman, K.G., et al. 2003. Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. *Genes & Dev.* **17**: 1352–1365.
- Perrino, C. and Rockman, H.A. 2006. GATA4 and the two sides of gene expression reprogramming. *Circ. Res.* **98**: 715–716.
- Phung, T.L., Ziv, K., Dabydeen, D., Eyiah-Mensah, G., Riveros, M., Perruzzi, C., Sun, J., Monahan-Earley, R.A., Shiojima, I., Nagy, J.A., et al. 2006. Pathological angiogenesis is induced by sustained Akt signaling and inhibited by rapamycin. *Cancer Cell* **10**: 159–170.
- Pipes, G.C., Creemers, E.E., and Olson, E.N. 2006. The myocardin family of transcriptional coactivators: Versatile regulators of cell growth, migration, and myogenesis. *Genes & Dev.* **20**: 1545–1556.
- Pouyssegur, J., Dayan, F., and Mazure, N.M. 2006. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* **441**: 437–443.
- Proud, C.G. 2005. eIF2 and the control of cell physiology. *Semin. Cell Dev. Biol.* **16**: 3–12.
- Puig, O. and Tjian, R. 2005. Transcriptional feedback control of insulin receptor by dFOXO/FOXO1. *Genes & Dev.* **19**: 2435–2446.
- Radimerski, T., Montagne, J., Rintelen, F., Stocker, H., van der Kaay, J., Downes, C.P., Hafen, E., and Thomas, G. 2002. dS6K-regulated cell growth is dPKB/dPI(3)K-independent, but requires dPDK1. *Nat. Cell Biol.* **4**: 251–255.
- Rakusan, K., Flanagan, M.F., Geva, T., Southern, J., and Van Praagh, R. 1992. Morphometry of human coronary capillaries during normal growth and the effect of age in left ventricular pressure-overload hypertrophy. *Circulation* **86**: 38–46.
- Rathmell, J.C., Elstrom, R.L., Cinalli, R.M., and Thompson, C.B. 2003. Activated Akt promotes increased resting T cell size, CD28-independent T cell growth, and development of autoimmunity and lymphoma. *Eur. J. Immunol.* **33**: 2223–2232.
- Reiss, K., Cheng, W., Ferber, A., Kajstura, J., Li, P., Li, B., Olivetti, G., Homcy, C.J., Baserga, R., and Anversa, P. 1996. Overexpression of insulin-like growth factor-1 in the heart is coupled with myocyte proliferation in transgenic mice. *Proc. Natl. Acad. Sci.* **93**: 8630–8635.
- Richey, P.A. and Brown, S.P. 1998. Pathological versus physiological left ventricular hypertrophy: A review. *J. Sports Sci.* **16**: 129–141.
- Rintelen, F., Stocker, H., Thomas, G., and Hafen, E. 2001. PDK1 regulates growth through Akt and S6K in *Drosophila*. *Proc. Natl. Acad. Sci.* **98**: 15020–15025.
- Rota, M., Boni, A., Urbanek, K., Padin-Iruegas, M.E., Kajstura, T.J., Fiore, G., Kubo, H., Sonnenblick, E.H., Musso, E., Houser, S.R., et al. 2005. Nuclear targeting of Akt enhances ventricular function and myocyte contractility. *Circ. Res.* **97**: 1332–1341.
- Rupnick, M.A., Panigrahy, D., Zhang, C.Y., Dallabrida, S.M., Lowell, B.B., Langer, R., and Folkman, M.J. 2002. Adipose tissue mass can be regulated through the vasculature. *Proc. Natl. Acad. Sci.* **99**: 10730–10735.
- Ruvinsky, I., Sharon, N., Lerer, T., Cohen, H., Stolovich-Rain, M., Nir, T., Dor, Y., Zisman, P., and Meyuhas, O. 2005. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes & Dev.* **19**: 2199–2211.
- Sadoshima, J. and Izumo, S. 1995. Rapamycin selectively inhibits angiotensin II-induced increase in protein synthesis in cardiac myocytes in vitro. Potential role of 70-kD S6 kinase

- in angiotensin II-induced cardiac hypertrophy. *Circ. Res.* **77**: 1040–1052.
- Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., Walsh, K., Schiaffino, S., Lecker, S.H., and Goldberg, A.L. 2004. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* **117**: 399–412.
- Sarbassov, D.D., Ali, S.M., and Sabatini, D.M. 2005a. Growing roles for the mTOR pathway. *Curr. Opin. Cell Biol.* **17**: 596–603.
- Sarbassov, D.D., Guertin, D.A., Ali, S.M., and Sabatini, D.M. 2005b. Phosphorylation and regulation of Akt/PKB by the rictor–mTOR complex. *Science* **307**: 1098–1101.
- Scheid, M.P. and Woodgett, J.R. 2001. PKB/AKT: Functional insights from genetic models. *Nat. Rev. Mol. Cell Biol.* **2**: 760–768.
- Schiekofer, S., Shiojima, I., Sato, K., Galasso, G., Oshima, Y., and Walsh, K. 2006. Microarray analysis of Akt1 activation in transgenic mouse hearts reveal transcript expression profiles associated with compensatory hypertrophy and failure. *Physiol. Genomics* **27**: 156–170.
- Schneider, V.A. and Mercola, M. 2001. Wnt antagonism initiates cardiogenesis in *Xenopus laevis*. *Genes & Dev.* **15**: 304–315.
- Schwertfeger, K.L., Richert, M.M., and Anderson, S.M. 2001. Mammary gland involution is delayed by activated Akt in transgenic mice. *Mol. Endocrinol.* **15**: 867–881.
- Schwertfeger, K.L., McManaman, J.L., Palmer, C.A., Neville, M.C., and Anderson, S.M. 2003. Expression of constitutively activated Akt in the mammary gland leads to excess lipid synthesis during pregnancy and lactation. *J. Lipid Res.* **44**: 1100–1112.
- Shibata, R., Ouchi, N., Ito, M., Kihara, S., Shiojima, I., Pimentel, D.R., Kumada, M., Sato, K., Schiekofer, S., Ohashi, K., et al. 2004. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat. Med.* **10**: 1384–1389.
- Shima, H., Pende, M., Chen, Y., Fumagalli, S., Thomas, G., and Kozma, S.C. 1998. Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. *EMBO J.* **17**: 6649–6659.
- Shioi, T., Kang, P.M., Douglas, P.S., Hampe, J., Yballe, C.M., Lawitts, J., Cantley, L.C., and Izumo, S. 2000. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. *EMBO J.* **19**: 2537–2548.
- Shioi, T., McMullen, J.R., Kang, P.M., Douglas, P.S., Obata, T., Franke, T.F., Cantley, L.C., and Izumo, S. 2002. Akt/protein kinase B promotes organ growth in transgenic mice. *Mol. Cell. Biol.* **22**: 2799–2809.
- Shioi, T., McMullen, J.R., Tarnavski, O., Converso, K., Sherwood, M.C., Manning, W.J., and Izumo, S. 2003. Rapamycin attenuates load-induced cardiac hypertrophy in mice. *Circulation* **107**: 1664–1670.
- Shiojima, I. and Walsh, K. 2002. Role of Akt signaling in vascular homeostasis and angiogenesis. *Circ. Res.* **90**: 1243–1250.
- Shiojima, I., Yefremashvili, M., Luo, Z., Kureishi, Y., Takahashi, A., Tao, J., Rosenzweig, A., Kahn, C.R., Abel, E.D., and Walsh, K. 2002. Akt signaling mediates postnatal heart growth in response to insulin and nutritional status. *J. Biol. Chem.* **277**: 37670–37677.
- Shiojima, I., Sato, K., Izumiya, Y., Schiekofer, S., Ito, M., Liao, R., Colucci, W.S., and Walsh, K. 2005. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J. Clin. Invest.* **115**: 2108–2118.
- Shiraishi, I., Melendez, J., Ahn, Y., Skavdahl, M., Murphy, E., Welch, S., Schaefer, E., Walsh, K., Rosenzweig, A., Torella, D., et al. 2004. Nuclear targeting of Akt enhances kinase activity and survival of cardiomyocytes. *Circ. Res.* **94**: 884–891.
- Skurk, C., Izumiya, Y., Maatz, H., Razeghi, P., Shiojima, I., Sandri, M., Sato, K., Zeng, L., Schiekofer, S., Pimentel, D., et al. 2005a. The FOXO3a transcription factor regulates cardiac myocyte size downstream of AKT signaling. *J. Biol. Chem.* **280**: 20814–20823.
- Skurk, C., Maatz, H., Rocnik, E., Bialik, A., Force, T., and Walsh, K. 2005b. Glycogen-synthase kinase3 β / β -catenin axis promotes angiogenesis through activation of vascular endothelial growth factor signaling in endothelial cells. *Circ. Res.* **96**: 308–318.
- Stanley, W.C., Recchia, F.A., and Lopaschuk, G.D. 2005. Myocardial substrate metabolism in the normal and failing heart. *Physiol. Rev.* **85**: 1093–1129.
- Stocker, H. and Hafen, E. 2000. Genetic control of cell size. *Curr. Opin. Genet. Dev.* **10**: 529–535.
- Stuckmann, I., Evans, S., and Lassar, A.B. 2003. Erythropoietin and retinoic acid, secreted from the epicardium, are required for cardiac myocyte proliferation. *Dev. Biol.* **255**: 334–349.
- Sun, J.F., Phung, T., Shiojima, I., Felske, T., Upalakalin, J.N., Feng, D., Kornaga, T., Dor, T., Dvorak, A.M., Walsh, K., et al. 2005. Microvascular patterning is controlled by fine-tuning the Akt signal. *Proc. Natl. Acad. Sci.* **102**: 128–133.
- Sun, H., Kerfant, B.G., Zhao, D., Trivieri, M.G., Oudit, G.Y., Penninger, J.M., and Backx, P.H. 2006. Insulin-like growth factor-1 and PTEN deletion enhance cardiac L-type Ca²⁺ currents via increased PI3K α /PKB signaling. *Circ. Res.* **98**: 1390–1397.
- Takahashi, A., Kureishi, Y., Yang, J., Luo, Z., Guo, K., Mukhopadhyay, D., Ivashchenko, Y., Branellec, D., and Walsh, K. 2002. Myogenic Akt signaling regulates blood vessel recruitment during myofiber growth. *Mol. Cell. Biol.* **22**: 4803–4814.
- Taniguchi, C.M., Emanuelli, B., and Kahn, C.R. 2006. Critical nodes in signalling pathways: Insights into insulin action. *Nat. Rev. Mol. Cell Biol.* **7**: 85–96.
- Taniyama, Y. and Walsh, K. 2002. Elevated myocardial Akt signaling ameliorates doxorubicin-induced congestive heart failure and promotes heart growth. *J. Mol. Cell. Cardiol.* **34**: 1241–1247.
- Taniyama, Y., Ito, M., Sato, K., Kuester, C., Veit, K., Tremp, G., Liao, R., Colucci, W.S., Ivashchenko, Y., Walsh, K., et al. 2005. Akt3 overexpression in the heart results in progression from adaptive to maladaptive hypertrophy. *J. Mol. Cell. Cardiol.* **38**: 375–385.
- Thom, T., Haase, N., Rosamond, W., Howard, V.J., Rumsfeld, J., Manolio, T., Zheng, Z.J., Flegal, K., O'Donnell, C., Kittner, S., et al. 2006. Heart disease and stroke statistics—2006 update: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* **113**: e85–e151.
- Trotman, L.C., Alimonti, A., Scaglioni, P.P., Koutcher, J.A., Cordon-Cardo, C., and Pandolfi, P.P. 2006. Identification of a tumour suppressor network opposing nuclear Akt function. *Nature* **441**: 523–527.
- Tschopp, O., Yang, Z.Z., Brodbeck, D., Dummmler, B.A., Hemmings-Mieszczak, M., Watanabe, T., Michaelis, T., Frahm, J., and Hemmings, B.A. 2005. Essential role of protein kinase B γ (PKB γ /Akt3) in postnatal brain development but not in glucose homeostasis. *Development* **132**: 2943–2954.
- Tuttle, R.L., Gill, N.S., Pugh, W., Lee, J.P., Koerberlein, B., Furth, E.E., Polonsky, K.S., Naji, A., and Birnbaum, M.J. 2001. Regulation of pancreatic β -cell growth and survival by the

- serine/threonine protein kinase Akt1/PKB α . *Nat. Med.* **7**: 1133–1137.
- Tzahor, E. and Lassar, A.B. 2001. Wnt signals from the neural tube block ectopic cardiogenesis. *Genes & Dev.* **15**: 255–260.
- Visconti, R.P., Richardson, C.D., and Sato, T.N. 2002. Orchestration of angiogenesis and arteriovenous contribution by angiopoietins and vascular endothelial growth factor (VEGF). *Proc. Natl. Acad. Sci.* **99**: 8219–8224.
- von Lewinski, D., Voss, K., Hulsman, S., Kogler, H., and Pieske, B. 2003. Insulin-like growth factor-1 exerts Ca²⁺-dependent positive inotropic effects in failing human myocardium. *Circ. Res.* **92**: 169–176.
- Wang, L., Ma, W., Markovich, R., Chen, J.W., and Wang, P.H. 1998. Regulation of cardiomyocyte apoptotic signaling by insulin-like growth factor I. *Circ. Res.* **83**: 516–522.
- Wolff, A.A., Rotmensch, H.H., Stanley, W.C., and Ferrari, R. 2002. Metabolic approaches to the treatment of ischemic heart disease: The clinicians' perspective. *Heart Fail. Rev.* **7**: 187–203.
- Wolfrum, C., Besser, D., Luca, E., and Stoffel, M. 2003. Insulin regulates the activity of forkhead transcription factor Hnf-3 β /Foxa-2 by Akt-mediated phosphorylation and nuclear/cytosolic localization. *Proc. Natl. Acad. Sci.* **100**: 11624–11629.
- Woodgett, J.R. 2005. Recent advances in the protein kinase B signaling pathway. *Curr. Opin. Cell Biol.* **17**: 150–157.
- Wullschlegel, S., Loewith, R., and Hall, M.N. 2006. TOR signaling in growth and metabolism. *Cell* **124**: 471–484.
- Yancopoulos, G.D., Davis, S., Gale, N.W., Rudge, J.S., Wiegand, S.J., and Holash, J. 2000. Vascular-specific growth factors and blood vessel formation. *Nature* **407**: 242–248.
- Yang, Z.Z., Tschopp, O., Di-Poi, N., Bruder, E., Baudry, A., Dummler, B., Wahli, W., and Hemmings, B.A. 2005. Dosage-dependent effects of Akt1/protein kinase B α (PKB α) and Akt3/PKB γ on thymus, skin, and cardiovascular and nervous system development in mice. *Mol. Cell. Biol.* **25**: 10407–10418.
- Yano, M., Ikeda, Y., and Matsuzaki, M. 2005. Altered intracellular Ca²⁺ handling in heart failure. *J. Clin. Invest.* **115**: 556–564.

Regulation of cardiac growth and coronary angiogenesis by the Akt/PKB signaling pathway

Ichiro Shiojima and Kenneth Walsh

Genes Dev. 2006, **20**:

Access the most recent version at doi:[10.1101/gad.1492806](https://doi.org/10.1101/gad.1492806)

References

This article cites 177 articles, 90 of which can be accessed free at:
<http://genesdev.cshlp.org/content/20/24/3347.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).