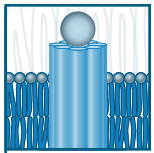


# MYOCARDIAL AKT: THE OMNIPRESENT NEXUS

Mark A. Sussman, Mirko Völkers, Kimberlee Fischer, Brandi Bailey, Christopher T. Cottage, Shabana Din, Natalie Gude, Daniele Avitabile, Roberto Alvarez, Balaji Sundararaman, Pearl Quijada, Matt Mason, Mathias H. Konstandin, Amy Malhowski, Zhaokang Cheng, Mohsin Khan, and Michael McGregor\*

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Sussman MA, Völkers M, Fischer K, Bailey B, Cottage CT, Din S, Gude N, Avitabile D, Alvarez R, Sundararaman B, Quijada P, Mason M, Konstandin MH, Malhowski A, Cheng Z, Khan M, McGregor M. Myocardial AKT: The Omnipresent Nexus. *Physiol Rev* 91: 1023–1070, 2011; doi:10.1152/physrev.00024.2010.—One of the greatest examples of integrated signal transduction is revealed by examination of effects mediated by AKT kinase in myocardial biology. Positioned at the intersection of multiple afferent and efferent signals, AKT exemplifies a molecular sensing node that coordinates dynamic responses of the cell in literally every aspect of biological responses. The balanced and nuanced nature of homeostatic signaling is particularly essential within the myocardial context, where regulation of survival, energy production, contractility, and response to pathological stress all flow through the nexus of AKT activation or repression. Equally important, the loss of regulated AKT activity is primarily the cause or consequence of pathological conditions leading to remodeling of the heart and eventual decompensation. This review presents an overview compendium of the complex world of myocardial AKT biology gleaned from more than a decade of research. Summarization of the widespread influence that AKT exerts upon myocardial responses leaves no doubt that the participation of AKT in molecular signaling will need to be reckoned with as a seemingly omnipresent regulator of myocardial molecular biological responses.

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## I. INTRODUCTION/BASICS OF AKT BIOLOGY

After decades of research, three vexing issues of cellular regulation continue to challenge cardiovascular biologists: growth, proliferation, and survival. In this respect, cardiovascular researchers share a similar obsession with cancer biologists who seek to influence the phenotypic behavior of transformed cells, with advances in understanding of oncogenic transformation repeatedly leading to profound in-

sights regarding myocardial cell biology. Such was the case over three decades ago when the gene first identified in association with rodent T-cell lymphoma as the product of transforming retrovirus AKT8 (603, 604) possessing homology with protein kinases A and C (PKA and PKC, respectively) (8) dubbed protein kinase B (PKB) that has come to be known as AKT kinase. Retrospectively, it is refreshing to look back at the relatively limited perspective of AKT functional activities in cell survival and proliferation from those early days of important discovery (13, 56, 71, 107, 351, 378, 450) and, with the benefit of hindsight, recognize that these scientists had found the proverbial “tip of the iceberg” with these initial studies. Subsequent years have produced a literal explosion of intellectual and practical understanding of molecular signal transduction in both normal and pathological conditions, with AKT serving as a canonical example of the complexity that lies beneath integration of signals for maintenance of homeostasis. However, the cancer and cardiovascular disciplines have adopted diametrically opposed perspectives on how to exploit the regulatory functions of AKT: whereas persistent cell survival and proliferation are the antitheses of what is needed to treat cancer, these same properties have often been the holy grail of cardiovascular biologists searching for ways to limit damage and promote repair in the wake of myocardial insults.

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The trifecta of cellular growth, proliferation, and survival lies at the crux of most, if not all, therapeutic interventional strategies to treat cardiovascular disease. Although manipulating these processes seems conceptually straightforward, this hypothetical goal has proven to be remarkably elusive in the myocardium. The challenges involved with this endeavor are readily illustrated by examining the legacy of literature documenting the relationship between AKT signal transduction and the myocardium. AKT serves as a critical nexus of integration between cellular stimuli and subsequent adaptive responses, and this pervasiveness of AKT participation had made it one of the most extensively characterized kinases in the myocardium. The substrates of AKT influence every aspect of cellular functions including not only growth, survival, and proliferation, but also metabolism, glucose uptake, gene expression, and cell-cell communication via initiation of paracrine and autocrine factor production. Owing to the enormity of information available for consideration, literature reviews typically concentrate on functional aspects of AKT biology in the context of a specific subtopic. With many such precedents providing excellent perspectives for additional information, readers of this review will be directed to those resources whenever possible. The distinguishing viewpoint of this treatise is an examination of AKT in the myocardial context by integrating a plethora of observations into a coherent perspective that will clarify how and why AKT has attained both celebrity and notoriety as a seemingly omnipresent node at the crossroads of myocardial cell biology.

## II. AKT IN THE MYOCARDIAL CONTEXT

### A. Survival

Evidence that serine/threonine kinases promote cell survival would seem indisputable at this point (132) as activation of these kinases is associated with pathogenesis of malignancies as well as resistance to apoptotic challenge that would otherwise limit dysregulated cell proliferation. Aside from oncogenic transformation, inhibition of these kinases also leads to increased damage in the wake of pathological challenge, indicating their role in normal cell persistence (25). Cellular survival induced by a plethora of cardioprotective agents converges on AKT activation. Subsequently, AKT activation leads to blockade of pro-apoptotic protein function and initiation of protective signaling cascades.

In the myocardial context, there is abundant evidence to support a cardioprotective role for AKT activation (138, 379, 458–460, 467). Preservation of cardiomyocytes and function is necessary for the heart. Several lines of evidence have shown the necessity of AKT signaling for cardiomyocyte, cardiac fibroblast, vascular smooth muscle cells (VSMCs), and endothelial cell survival (522). Insulin-like growth factor I (IGF-I) activates upstream phosphatidylinositol 3-kinase (PI3K), resulting in the activation of AKT and

multiple downstream effectors. AKT activation reduced apoptotic cardiomyocyte death in response to ischemia-reperfusion injury (26, 32), pressure overload challenge (84), and oxidative stress (12). Declining AKT activity is also linked to increased apoptosis in pacing-induced heart failure (18). However, viral myocarditis may diverge from the generally cardioprotective role for AKT, as inhibition of AKT activity seems to improve protective effects (182–184).

Many downstream targets of AKT have been shown to contribute to its pro-survival effects such as phosphorylation of BCL-2 family members (251, 301, 324), activation of Forkhead transcription factors (242, 406, 619), increase in nitric oxide (NO) (155, 275, 276, 518), regulation of  $\text{Ca}^{2+}$  cycling (103, 119, 349), and cardiac stem cell survival (632, 643). Activation of AKT has been shown to modulate pro-apoptotic proteins through the phosphorylation of BCL-2 family members BAX and BAD. During stress or injury, BAX will translocate to the mitochondria and permeabilize the membrane-forming pores, thus allowing for cytochrome *c* release, and jeopardizing the stability of the mitochondria. Phosphorylation of BAX, at serine 184, by AKT prevents BAX translocation to the mitochondria through a conformational change (642). Phosphorylation of BAD at serine 136 releases BCL-xL from BAD, allowing it to perform its anti-apoptotic effects (12, 334).

Forkhead transcription factor, FOXO3a, is involved in the regulation of the cell cycle by upregulating the transcription of death receptor ligands, including the regulation of FasL and TRAIL gene expression. Furthermore, Forkhead transcription factors have recently been shown to upregulate the expression of BIM. BIM is a BCL-2 family member that initiates mitochondrial dysfunction leading to apoptosis. Phosphorylation of FOXO3a by AKT in the nucleus results in FOXO3a nuclear exclusion and transport into the cytosol in an inactive state, resulting in a reduction of apoptosis (78).

Endothelial NO synthase (eNOS) is responsible for the production of NO. eNOS-derived NO serves important functions within the heart including ventricular relaxation, myocardial remodeling, regulation of VMSC proliferation, etc. The release of NO has been shown to be mediated through the PI3K/AKT pathway through engagement of membrane estrogen receptors and without an increase in intracellular  $\text{Ca}^{2+}$  to keep cardiac homeostasis (276). Activation of AKT during preconditioning leads to phosphorylation of eNOS and is essential for cardioprotection (270, 660).

### B. Proliferation

As an oncogenic protein, it is no surprise that AKT promotes proliferation in the context of cancer. On the other hand, cardiomyocytes are notoriously resistant to onco-

genic transformation and mitotic activity. Cardiomyocyte proliferation occurring primarily during prenatal and early postnatal development decreases shortly after birth. Neonatal cardiomyocytes can grow by increases in both cell number (proliferation) as well as cell size (hypertrophy), but adult cardiomyocytes grow predominantly by hypertrophy, with proliferation being identified at very low levels (44, 45, 338). Within the past decade, genetic manipulation has been utilized to induce cardiomyocyte proliferation and DNA synthesis by overexpressing cell cycle mediators (cyclin D, cyclin A, cyclin B, Cdk2), growth factors (IGF-I, FGF2), transcription factors (c-Myc, E2F2), and knockout of cell cycle inhibitors (p27, Rb; reviewed in Refs. 11, 53, 529). Around this time, factors that influence cardiomyocyte cell cycle reentry were also being identified. IGF-I, a potent activator of AKT, increases kinase activity of cyclin D/E/A and induces DNA synthesis in adult cardiomyocytes (549, 550). Transgenic overexpression of IGF-I results in a progressive increase in the number of cells in the heart without influencing myocyte volume (547). FGF1 stimulation and p38 inhibition promote cytokinesis in adult cardiomyocytes through a PI3K/AKT-dependent pathway (178). Combined administration of FGF1 and p38 mitogen-activated protein (MAP) kinase inhibitor increases cardiomyocyte mitosis and improves cardiac function after myocardial infarction (177). Platelet-derived growth factor (PDGF)-induced neonatal cardiomyocyte proliferation correlates with AKT activation leading to inactivation of glycogen synthase kinase  $3\beta$  (GSK- $3\beta$ ) and downregulation of p27 (287). Periostin, a component of the extracellular matrix associated with epithelial-mesenchymal transition during cardiac development, induces cell-cycle reentry of adult cardiomyocytes by activation of AKT but not ERK1/2 (376). Overexpression of the phosphatase PTEN or treatment with LY294002 abrogates periostin-induced DNA synthesis and cell cycle reentry. Conversely, other studies show periostin is critical for regulation of hypertrophic responses (516) rather than proliferation (431) following pressure overload and myocardial infarction. Neuregulin1 induces adult mononucleated cardiomyocytes to divide by signaling through tyrosine kinase receptor ErbB4 to activate the PI3K/AKT pathway (47).

Several downstream targets of AKT regulate cardiomyocyte proliferation during development. Deletion of GSK- $3\beta$  induces cardiomyocyte hyperproliferation associated with increased expression of GATA4, cyclin D1, and c-Myc (353). Myocardial specific transgenic expression of FOXO1 decreases myocyte proliferation during heart development by premature activation of p21, p27, and p57 (186). IGF-I stimulation/AKT overexpression promotes embryonic cardiomyocyte proliferation and cytoplasmic localization of FOXO (186, 503, 595). Nuclear-targeted AKT expression also produces a hypercellular phenotype (558) characterized by increased cardiomyocyte cycling and expansion of the cardiac progenitor cell (CPC) population (244). Consis-

tent with observations of myocardial hyperplasia, in the presence of periostin, nuclear targeted AKT doubles the number of BrdU-positive cardiomyocytes (376). These findings are in agreement with observations of increased AKT activity correlating with proliferation of cardiomyocytes (287, 465), downregulation of AKT upon differentiation (337), and requirement of PI3K-dependent signaling in proliferation (342). Overexpression of NOTCH induces phosphorylation of AKT and proliferative signaling in neonatal and adult cardiomyocytes (75, 110, 245). During pathological challenge, upregulated levels of AKT (15, 60) correlate with increased abundance of c-KIT-positive CPCs (201, 373). These CPCs are maintained through AKT/GSK- $3\beta$  signaling, because inhibition of AKT impairs CPC proliferation, whereas inhibition of GSK- $3\beta$  enhances their growth (624). Collectively these results support the premise that PI3K/AKT signaling plays a critical role in proliferation of both cardiomyocytes and CPCs.

### C. Metabolism

Metabolism and AKT are inextricably linked even through diet, as shown by multiple and divergent threads of investigation. Stimulation of glucose uptake triggers activation of AKT downstream of PI3K (161). AKT activity is tied to glycolytic metabolism, with reduced glycolysis prompting reduction of AKT phosphorylation and cardiomyopathic consequences (162). Impaired AKT activity is also a common feature of altered signaling associated with diabetic cardiomyopathy (167). In comparison, undernutrition results in compensatory increases in AKT activity associated with hyperinsulinemia (224). High cholesterol-fructose alters induction of AKT signaling through enhanced insulin resistance and provokes cardiomyopathic disease (146). Along similar lines, AKT activity is stimulated in response to a high-fat diet resulting in obesity and increased stress (159). Peroxisome proliferator-activated receptor (PPAR)- $\gamma$  is one member of a family of nuclear receptor transcription factors regulating metabolism at the gene expression level that influences AKT activity with ties to hypertrophic remodeling, hypertension, and diabetes (168, 185, 309–311, 412, 440, 527, 685). Supplementation of diet with omega-3 polyunsaturated fatty acids (omega-3 PUFA) purported to reduce the risk of heart failure leads to increased AKT expression, although activity was maintained at constant levels (169). Dietary supplementation with red palm oil improves recovery from ischemia-reperfusion injury in rats associated with increased AKT phosphorylation (179).

AKT exerts this central role in regulating heart metabolism by direct or indirect interaction with key regulatory molecules controlling glucose transporter 4 (GLUT4) (62), FOXO proteins transcriptional activity (636), mTOR pathway (523), GSK- $3\beta$  (recently reviewed in Ref. 449), and mitochondrial function (483, 617) as discussed later in this review.

## 1. AKT and GLUT4

The heart normally derives energy from oxidation of fatty acids (FA) (60–70%), glucose (30–40%), and lactate (10%) (430). However, glucose oxidation has a central role in energy metabolism of the heart. Obesity and diabetes, two of the most important risk factors for development of cardiomyopathy, are associated with reduced utilization of glucose and increased oxidation of FA and lactate (64, 89, 659), concomitantly to impaired insulin-dependent AKT activation (646). In cardiomyocytes, glucose metabolism is triggered by transport through the membrane mediated through GLUT1 and GLUT4 glucose transporters localized in the sarcolemma and intracellular membrane compartments, respectively. GLUT1 is implicated in maintenance of glucose homeostasis under basal conditions, whereas GLUT4 translocates to the sarcolemma and transverse tubule membranes in response to normal and pathological stimuli (134, 190). Decreased glucose utilization and increased fatty acid consumption caused by diet-induced obesity correlates with reduced expression of GLUT4, which precedes the impairment of insulin-dependent AKT activation (667). Impairment of insulin-stimulated AKT/GLUT4 signaling parallels ventricular contractile dysfunction and increased mortality rate of streptozotocin-induced diabetic rats subjected to ischemia-reperfusion treatment (307). AKT also drives GLUT4 translocation to the sarcolemma under oxidative stress condition in cardiomyocytes, and also following ischemia in conjunction with AMP-activated protein kinase (AMPK; Ref. 297). AKT promotes GLUT4 translocation to the sarcolemma by phosphorylating and inactivating AKT substrate 160 (AS160), thereby inhibiting Rab function and favoring GLUT4 translocation in adipocytes and muscle (663). Importance of GLUT4 translocation under pathological conditions is demonstrated by the fact that its activation is the major mechanism by which the heart increases glucose uptake during ischemia (612). So too, chronic cardiac-specific overexpression of activated AKT increases basal glucose uptake and glycogen deposition while inhibiting the response to insulin (457). Cardiac-selective GLUT4 deficiency leads to profound and irreversible systolic and diastolic dysfunction after ischemia and reperfusion in mice (629). In summary, the antiapoptotic effect of insulin following ischemic reperfusion injury is mostly mediated by PI3K/AKT pathway (213), pointing directly toward the protective effect of AKT being influenced by and inextricably tied to glucose metabolism (670).

## 2. AKT and FOXO

Another emerging pathway through which AKT influences metabolism is by regulating translocation and activity of the forkhead transcription factors (FOXO) subfamily that includes FOXO1, FOXO3a, and FOXO4, which are directly phosphorylated by AKT. FOXO transcription factors participate in control of energy metabolism by regulating insulin signaling and glucose and lipid metabolism

(242), although most of the literature regarding FOXO proteins is based on experiments performed on noncardiac cells. For example, in the liver, AKT inhibits gluconeogenesis by blocking FOXO-mediated transcription of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (181). However, new experimental evidence proves a central role for the FOXO protein family in the cardiac context as well (556). FOXO1 and FOXO3a expression increases and accumulates in the nucleus during heart development concomitant with cyclin kinase inhibitors (CKIs), p21<sup>CIP1</sup>, and p27<sup>KIP1</sup>, inducing cell cycle withdrawal in cardiomyocytes after birth (186). Cardiac-restricted overexpression of wild-type or dominant negative FOXO1 induces embryonic lethality at E10.5 and abnormal morphology of the myocardium by embryonic day 18.5, respectively. These phenotypes are related to premature induction or prolonged suppression of CKIs in the heart and intimates that the PI3K/AKT/FOXO pathway has a central role in heart development (186). In postnatal heart the PI3K/AKT/FOXO axis regulates cardiomyocyte size, with increased phosphorylation levels of AKT and FOXO3a associated with cardiac hypertrophy in vivo, while FOXO3a overexpression reduces IGF-I-mediated hypertrophic effects and decreases cardiomyocyte size in vivo (595). Sustained FOXO proteins overexpression in cardiomyocytes leads to increased AKT phosphorylation and kinase activity without influencing other signaling pathways such as p38, ERK, or JNK (503). AKT and FOXO proteins are also apparently connected through atrogin-1 (a direct target gene of FOXO3a) and the phosphatases PP2A and calcineurin, the latter proposed to target AKT directly (503). Transcriptional induction of atrogin-1 proteasomal factor reduces PP2A and calcineurin phosphatase activity as well as interaction with AKT. The physiological result of this sustained activation is an attenuated insulin response in cardiomyocytes (503).

## 3. AKT and mTOR

AKT influences protein synthesis through acting upon several translation factors and ribosomal proteins. AKT phosphorylates and inactivates tuberous sclerosis factor 2 (TSC2), thereby inducing formation of active Rheb which, in turn, phosphorylates and activates the mammalian target of rapamycin (mTOR) (318, 542), which is central to protein synthesis and cell growth. Activated mTOR targets 4E-binding protein-1 (4E-BP1) and p70 ribosomal S6 protein kinase (p70S6K) (543). Phosphorylation of 4E-BP1 by mTOR is necessary to ablate its inhibitory function on the eukaryotic initiation factor 4E (eIF-4E), thus promoting the initiation step of protein synthesis. Concurrent activation of p70S6K phosphorylates S6 ribosomal protein that is involved in the regulation of protein translation. Downstream of p70S6K is the eukaryotic elongation factor-2 (eEF2), which upon phosphorylation is inactivated, promoting protein elongation (543).

The relationship between insulin or IGF-I-mediated AKT activation and cardiac cell growth (119, 471) depends on mTOR activation. Specifically, insulin induces TSC2 phosphorylation in adult ventricular cardiomyocytes (555), and the physiological hypertrophic response of NRCMs to T3 thyroid hormone is associated with mTOR activation mediated by AKT (350). In addition, rapamycin attenuates heart overgrowth in transgenic mice overexpressing constitutively activated AKT specifically in the heart (584). Another mechanism by which AKT influences mTOR pathway is associated with the phosphorylation of the proline-rich AKT substrate of 40 kDa (PRAS40), a recently identified mTOR regulator. Once phosphorylated by AKT, PRAS40 binds to 14-3-3, thereby relieving PRAS40-induced inhibition of mTOR and allowing its action on p70S6K (565, 652). In the heart, insulin activates mTOR through the AKT/PRAS40 pathway, while leucine, another strong inducer of mTOR, elicits PRAS40 phosphorylation by a pathway directly dependent on PDK1 activation (543). Although still poorly characterized, the connection between the AKT and mTOR pathways represents a novel entry point for molecular intervention to regulate myocardial hypertrophy and remodeling.

#### D. Growth/Hypertrophy

By virtue of participation as a nodal kinase in facilitating cellular metabolism and remodeling, AKT has long been recognized as a pivotal participant in hypertrophic signaling (163, 254, 278, 396, 434). Interestingly, AKT expression decreases during pregnancy and normalizes during the post-partum period, suggesting AKT plays an antihypertrophic role in physiological hypertrophy (237). The developmental growth and physiological hypertrophy mediated by AKT signaling stem from upstream induction via class I(A) PI3Ks (435). AKT phosphorylation levels show temporal changes in exercised rats, decreasing at 1 wk and increasing selective phosphorylation of Ser-473 at 3 wk (239). AKT activity is induced by treatment of neonatal rat cardiomyocytes with TNF- $\alpha$ , leading to increased protein synthesis and cellular hypertrophy (290).

Thyroid hormones regulate physiological cardiac hypertrophy acting both as transcriptionally active proteins while also participating in cytoplasmic-initiated signaling processes (210). Thyroid hormones activate PI3K/AKT in cardiomyocytes, which in turn induces the mTOR pathway and increases protein translation (350). Activation/inactivation of AKT/mTOR pathway seems to be related to development of physiological adaptive versus pathological cardiac hypertrophy. Mice subjected to either treadmill training for 6 wk or transverse aortic constriction (TAC) developed physiological versus pathological cardiac hypertrophy associated with activation versus inhibition of the AKT/mTOR signaling pathway (348, 589). Thus the PI3K/AKT axis seems more linked to physiological hypertrophy,

whereas MAPK signaling, in collaboration with the PKC and calcineurin/NFAT pathways, participates in the development of the pathological hypertrophy typically induced by angiotensin II (469). AKT also controls cardiomyocyte size by inactivating the FOXO transcription factors that promote the expression of atrophic genes (595). Importantly, recent data suggest that the deregulation of the AKT/FOXO axis can be associated with the development of pathological hypertrophy (407). These results confirm the idea that AKT-dependent hypertrophic heart *in vivo* is associated with hyperphysiological levels of kinase activity in the cytoplasm resulting in a deregulation of AKT upstream and downstream targets (114, 455, 498, 584). Interestingly, our group demonstrated that AKT localization is crucial to regulating function (347, 562). Overexpression of nuclear targeted AKT enhances cardioprotection and antagonizes cardiac hypertrophy (590, 641).

#### E. Remodeling/Regeneration/Repair

Alterations in AKT activity level are linked to the “reverse remodeling” observed following initiation of left ventricular assist device (LVAD) support in patients suffering from heart failure (27). Decreases in the PI3K/AKT pathway are likely to contribute to molecular changes in aging myocardium associated with enhanced susceptibility to cell death (85). Increased AKT phosphorylation is also associated with exercise (367, 368). Collectively, these observations indicate the central role AKT plays in cardiac remodeling.

Within the last few decades, research into cardiac regeneration has gained traction and paved the way for development of potential therapies targeting cardiac repair following pathological insult. As a prosurvival and proliferative cardiac signal, not surprisingly, the PI3K/AKT pathway participates in almost every aspect of cardiac regeneration. The following sections present various roles of AKT in angiogenesis, myocyte renewal, stem cell activation, and cell based therapies.

##### 1. AKT role in vasculogenesis

As a downstream effector of various angiogenic cytokines and growth factors, AKT is frequently identified as the mechanism underlying cytoprotection and neovascularization conferred by these agents. For example, AKT is thought to mediate the beneficial effects of statins applied to a model of hindlimb ischemia, enhancing proliferation, migration, and survival of bone marrow-derived EPCs (381, 426). Conversely, knockdown of PI3K $\gamma$  results in impaired neovascularization and endothelial progenitor function in ischemic hindlimb muscles (439). Cardioprotective effects of the traditional Chinese medicine shu-mai-tang include angiogenesis and arteriogenesis and are thought to be mediated via PI3K/AKT signaling (682). Exogenous nerve growth factor supports angiogenesis and myocyte survival

in infarcted murine hearts via the AKT/FOXO pathway (78, 475). CD151 induces endothelial cell proliferation, migration, and neovascularization in infarcted hearts via PI3K/AKT activation (695, 696), while periostin signals through FAK and AKT to mediate recruitment of activated cardiac fibroblasts to sites of cardiac injury following acute myocardial infarction (581). Intracardiac injection of SDF-1a into infarcted mouse heart improves cardiomyocyte survival and increases neoangiogenesis, potentially via activation of AKT (572). VEGF2-treated EPCs have enhanced AKT activation, and infarcted hearts receiving these VEGF-2 treated EPCs exhibit improved angiogenesis and cardiac function compared with control treated hearts (582), while inhibition of AKT by Ox-LDL impairs endothelial differentiation in bone marrow stem cells (102). Collectively, these studies indicate a pivotal role for PI3K/AKT signaling in vasculogenesis following cardiac injury.

## 2. AKT role in myocyte renewal and stem cell activation

Cardiac stem cells express IGF-I receptor and the IGF-I ligand, rendering them responsive to growth factor treatment in the infarcted myocardium. Stimulation with IGF-I activates AKT in these cells, promoting proliferation and survival, and thereby enhancing cardiac repair (644). IGF-I overexpression in murine heart increases activation of AKT, improves cardiomyocyte survival and renewal, and boosts the population of cardiogenic c-KIT<sup>+</sup> progenitor cells. Additionally, studies applying nanofibers coated with IGF-I to infarcted myocardium alone or in combination with adoptively transferred cardiac progenitor cells demonstrate improved survival and regeneration of myocytes and vessels in conjunction with AKT activation (136, 524, 633). Postnatal cardiac myocyte proliferation is extended and progenitor cell cycling enhanced in hearts of mice engineered to overexpress cardiac specific nuclear-targeted AKT (244). Similarly, PIM1, identified as a mediator of cardiac protection downstream of AKT, also promotes cardiac myocyte and progenitor proliferation in hearts of mice engineered to overexpress cardiac specific PIM1 (117).

Cardiac c-KIT<sup>+</sup> precursor cells expressing AT2 receptors may trigger AKT and STAT3 survival signaling in damaged myocardium (15). Likewise, cultured rat postinfarct cardiac c-KIT<sup>+</sup>/estrogen receptor (ER) $\alpha$  cells exhibit increased gene expression of AKT and enhance myocyte survival in coculture with adult rat cardiomyocytes (60).

## 3. AKT cross-talk with developmental/stem cell signaling pathways

AKT has been shown to activate and be activated by stem cell signaling proteins such as NOTCH and sonic hedgehog (19) and may contribute to the cardioprotective mechanism underlying their regenerative activity in the

heart (245). Treatment of infarcted hearts with SHH gene therapy improves cardiac function and upregulates expression of cytokines upstream of PI3K/AKT signaling, notably IGF-I and VEGF, in cardiac fibroblasts (382). PI3K/AKT may also mediate cardiomyocyte differentiation by canonical WNT by suppressing GSK-3 $\beta$  activity. Conversely, AKT counteracts profibrotic canonical WNT signaling during cardiomyogenesis and postinjury repair (482, 499).

## 4. Paracrine effects of exogenous progenitor cells

Cell-based therapy has emerged as an exciting frontier for the treatment of heart disease. Numerous laboratories are now investigating the reparative potential of various cells types, such as mesenchymal stem cells (MSCs), CPCs, or embryonic stem cells (ESCs). Varying degrees of functional benefit are documented depending on the model system and cell type used, and a key question remains as to whether cell engraftment or paracrine effects of the adoptively transferred cells are responsible for the improvement in cardiac function over control treated hearts.

Adoptive transfer of cardiosphere-derived human cardiac progenitor cells increases AKT protein levels in the infarct region and border zone of recipient mouse hearts. The authors measure the proportion of cardioprotection derived from paracrine effects versus direct regeneration and conclude that both mechanisms contribute to the cardiac improvement observed (98).

Bone marrow-derived MSCs engineered to overexpress AKT repair infarcted myocardium better than lacZ expressing control cells. Subsequent studies claim that paracrine effects, namely, secretion of growth factors and cytokines that promote survival and proliferation, account for cardiac benefits bestowed by these cells. Most recently, secreted frizzled related protein 2 (SFRP2) has been identified as a specific paracrine factor generated by AKT-overexpressing MSCs. SFRP2 acts by inhibiting the pro-apoptotic actions of canonical WNT3a signaling in cardiac myocytes subjected to hypoxia/reoxygenation injury (231–233, 446, 482, 509, 694). Interestingly, IGF-I overexpressing MSCs exhibit paracrine activity and enhanced engraftment when adoptively transferred into infarcted rat myocardium. IGF-I MSCs stimulate activation of AKT in recipient hearts as well as secretion of SDF-1a, which mobilizes and attracts endogenous bone marrow stem cells. Levels of phosphorylated AKT are increased in SDF-1a-treated MSCs, while inhibition of PI3K/AKT prevents SDF-1a/CXCR4-dependent migration of MSCs (257, 684).

Conversely, c-KIT<sup>+</sup> bone marrow-derived stem cells lacking AKT1 perform poorly compared with their wild-type counterparts; intravenously injected “armed” wild-type stem cells restore ventricular function, promote angiogenesis, and are retained for at least 2 wk in infarcted mouse

hearts, whereas application of “armed” AKT-deficient stem cells confer nominal cardiac benefit if any (639).

## F. Aging

Cellular senescence contributes to the decline of cell function during aging. The loss of pro-survival signaling and increased cellular senescence leads to declining function of the heart in old age. The connection between aging and diminution of IGF-I signaling eventually led to examination of AKT-mediated signaling as the critical hub of age-related heart disease (340). Loss of AKT activity correlates with diminished proliferation and development of a senescent phenotype in cardiac fibroblasts (153). Correlates of the aging phenotype are reduced insulin sensitivity and cardiac dysfunction associated with reductions in AKT expression and phosphorylation levels (188, 189).

Oxidative stress contributes a great deal to age-related diseases due to the accumulation of reactive oxygen species. The decrease in survival signaling through AKT leads to sensitivity in ROS-induced apoptosis in the heart, along with many other cell types (313, 316). Decrease in IGF-I signaling decreases CSC division leading to the decrease of functionally competent CSC reserves and the potential of regenerating new myocytes (633). Antagonizing IGF-I and blunted AKT expression leads to the upregulation of pleiotrophin during myocardial infarction as well as dilated cardiomyopathy resulting in an increase of apoptosis (410). Furthermore, exacerbated reperfusion injury in aged female hearts is correlated with blunted AKT activation (313).

AKT-mediated signaling has been shown to act upon different mediators of senescence. Specific cellular proteins correlated with the induction of senescence include p16, p21, p27, and p53. Accumulation of p16 in aged mice is representative of cellular senescence. However, in IGF-I transgenic mice, which have a consistent activation of PI3K/AKT, expression of p16 is blunted in older ages, allowing the assembly of cyclin D and CDK4/6 complexes to form uninterrupted for G<sub>1</sub> to S phase transition. AKT has been shown to inhibit p21 through phosphorylation on two sites and allowing for sustained cellular proliferation (413, 697). p27 has been shown to inhibit G<sub>1</sub> phase cyclins and CDKs causing cell cycle arrest. The presence of AKT has been shown to phosphorylate p27 on multiple sites, including Thr-198 (205). Phosphorylation of p27 on Thr-198 by AKT promotes binding of 14-3-3 and its cytoplasmic localization and eventually degradation of p27 (205). AKT has been shown to phosphorylate and activate MDM2 ubiquitination activity. Levels of p53 protein are decreased with the presence of AKT through increased ubiquitination of p53 by MDM2 (511).

Aging that prompts downregulation of VEGF, and presumably downstream AKT signaling as well, is blunted

by exercise training (313). Aerobic exercise leads to an increase in insulin signaling which activates the AKT/mTOR pathway and enhances muscle protein synthesis (206). AKT has a phosphorylation consensus target sequence within mouse telomerase, and increasing amounts of nuclear AKT increase telomerase activity (633). Furthermore, age-related alterations in AKT expression affect eNOS phosphorylation which, in turn, increases risk of age-associated hypertension (596). Multiple lines of evidence show an increase in AKT phosphorylation on a caloric restricted diet in the heart as well as hepatocytes (1, 316, 428).

## G. AKT Isoforms: AKT1 Versus AKT2

Mammalian cells contain three genes that encode for three isoforms of AKT, termed AKT1 (PKB $\alpha$ ), AKT2 (PKB $\beta$ ), and AKT3 (PKB $\gamma$ ). The three isoforms are highly related to each other and are activated by shared pathways via PI3K. All isoforms are expressed in the heart, but AKT1 and AKT2 are the most abundant isoform in the myocardium (459). The distinctions of effects mediated between AKT isoforms add layers of complexity to the delineation of AKT-mediated effects in the myocardium. The advent of genetically engineered AKT knockout models has empowered assessment of the roles played by AKT isoforms in myocardial biology and revealed distinct functions for each protein. In mouse models of global deficiency of AKT1, diminished somatic growth is observed, while AKT2 deficiency causes insulin resistance and diabetes mellitus (100, 101), indicating that AKT2 plays a key role in glucose metabolism. The latter is further confirmed by the existence of a family with an inherited missense mutation in the AKT2 gene, the phenotype of which is associated with severe insulin resistance and diabetes (226). Knockout of AKT3 reduces brain size but has no effects on growth or metabolism (171), whereas cardiac specific transgenic overexpression of AKT3 in the heart leads to maladaptive hypertrophy (622). Phenotypes of mice with ablation of AKT isoforms are summarized in **TABLE 1**. Studies comparing AKT1 and AKT2 (as the most abundant physiological isoforms in the heart) reveal the split personality of AKT isoforms participating in “physiological” versus “pathological” hypertrophic remodeling (140, 141, 496). Several studies have proven the cardioprotective role of AKT1 in response to pathological challenges, and the impact of AKT1 activity is predominantly in the realm of physiological cardiac growth and antagonized pathological remodeling. Conversely, the loss of AKT1 in this context leads to exacerbated hypertrophic responses consistent with a role for AKT blunting hypertrophy, similar to effects noted for nuclear accumulation of AKT (641). In contrast, AKT2 is dispensable in the development of cardiac hypertrophy in response to physiological or pathological stimuli, but is primarily involved in insulin-stimulated glucose uptake and metabolism as well as cellular survival in response to ischemic injury.

**Table 1** Phenotypic effects on the heart for genetic manipulation of AKT

Genotype	Phenotype	Reference Nos.
AKT1 GKO	Viable, reduced size of organs, decreased survival after cardiomyopathic injury.	92, 141
AKT2 GKO	Normal cardiac phenotype. Insulin resistance, diabetes, pancreatic $\beta$ -cell failure.	100, 222
AKT3 GKO	Neurological phenotype, reduced brain size.	173
Cardiac AKT1 TG	Cardiac hypertrophy. Increased cardiomyocyte cell size.	114, 455, 584
Nuclear AKT1 TG	Increased cell number, decreased cell size, increased contractile function.	558, 590
Cardiac AKT3 TG	Maladaptive hypertrophy.	622

### III. AKT SIGNALING IN THE MYOCARDIUM

#### A. Upstream Inductive Signals: Hormones, Cytokines, Drugs, Dietary Agents, Enzymes, Integrins, and Others

The mechanism of AKT activation in the heart and other systems has been well reviewed in several publications (459, 610). The binding of a ligand (hormone, cytokine, integrin, peptide, or small molecule) causes cell surface receptor intracellular domain phosphorylation (receptor tyrosine kinase, RTK) or receptor conformational change (G protein-coupled receptor, GPCR). The SH2 domain of the p85 subunit of PI3K binds to the activated receptor, bringing the complex into close association with the cell membrane or cardiomyocyte sarcolemma. The p110 catalytic subunit of PI3K catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), which is embedded in the cell membrane, to phosphatidylinositol 3,4,5-trisphosphate (PIP<sub>3</sub>). Both AKT and 3-phosphoinositide-dependent protein kinase [PDK1, (or PDPK1)] contain a pleckstrin homology (PH) domain which will bind to PIP<sub>3</sub> at the cell membrane, bringing the two kinases into close association with the plasma membrane and, hence, each other. AKT is a substrate of the constitutively active kinase PDK1 and will be phosphorylated at serine-473 and tyrosine-308 as well as other sites when the two proteins interact. Active, phosphorylated AKT is then liberated from the sarcolemma and can migrate to different cellular compartments to phosphorylate substrate molecules (FIGURE 1).

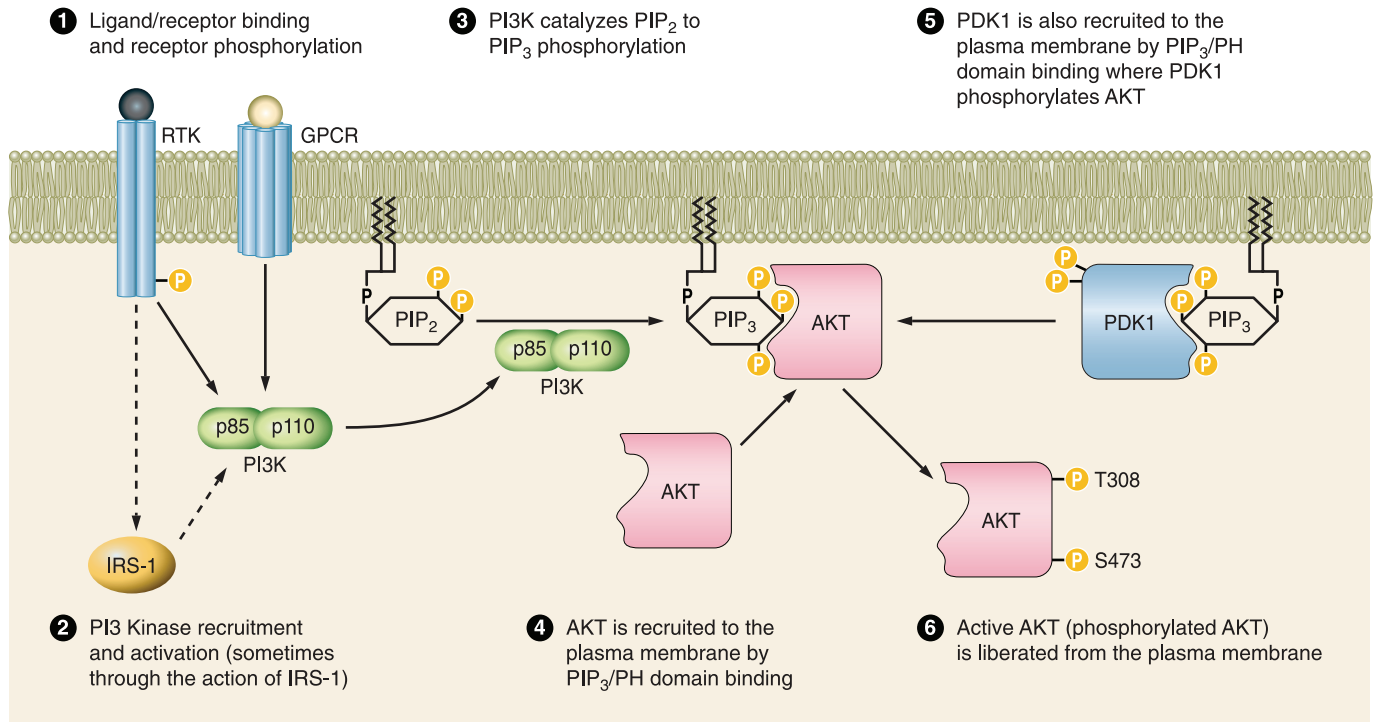
A multitude of cardioprotective factors exert their anti-apoptotic action, at least in part, in conjunction with AKT activation. These factors are diverse in nature and can be categorized as hormones, cytokines, integrins, drugs/small molecules, nutrients, as well as others. These categories may overlap and are subject to interpretation. However, here, for the sake of clarity, hormones are generally systemic actors (endocrine) and cytokines are local actors (paracrine/autocrine). The list of AKT acti-

vators described below is representative but by no means exhaustive. See TABLE 2 for a summary of the upstream AKT activators, receptors, and their respective reported effects.

Hormones and cytokines are the classically described activators of AKT signaling including the following: adrenomedullin (675), angiotensin II (ANG II) (128, 130, 228, 285), atrial natriuretic peptide (ANP) (347), erythropoietin (359, 515, 635), estrogen (306, 532, 534), ghrelin (33), growth hormone (GH) (432), insulin (12, 48, 213), resistin (214), thyroid hormone (350, 386, 387), angiotensin (122), cardiotrophin (59, 385, 486), granulocyte colony-stimulating factor (G-CSF) (415, 486), IGF-I (158, 298, 411, 672), interleukin-18 (88, 112), leukemia inhibitory factor (LIF) (289, 502), neuregulin-1 (207, 404, 631), PDGF (286, 300), stromal cell-derived factor 1 (SDF-1 $\alpha$ ) (302, 572), urocortin (58), and WNT1-induced secreted protein-1 (WISP1) (113). Small signaling peptides bradykinin (108, 478), endothelin (577), and secreted thymosin  $\beta$ 4 activate (54) AKT via indirect mechanisms. A majority of the hormone and cytokine factors act through AKT to induce hypertrophy or repress apoptosis.

Ingestion of compounds such as pharmacological agents and nutritional supplements can also activate AKT. Some small molecules that are available on the legitimate or not-so-legitimate market that have been shown to activate AKT in the context of the heart are as follows: acetylcholine (339, 371), adenosine or adenosine-like agonists (227),  $\beta$ 2 adrenergic agonists (96), cannabinoids (284), eplerenone (363), phenylephrine (106), rosiglitazone (356, 692), and the statin-class molecules (268, 417). Isoflurane and related compounds (324, 557, 698) as well as morphine (243) have been shown to activate AKT in conjunction with anesthesia-induced cardioprotection. There is an abundance of research on investigational new classes of compounds, some of which are from unexpected sources. Interestingly, two compounds from pathogenic agents mediate cardiomyocyte survival in part via activation of AKT, a *Trypano-*





**FIGURE 1** Upstream AKT signaling. Schematic diagram representing the receptor-mediated phosphorylation and activation steps required for the ultimate phosphorylation and activation of AKT. GPCR, G protein-coupled receptor; RTK, receptor tyrosine kinase; IRS-1, insulin receptor substrate 1; PI3K, phosphoinositide 3-kinase; PDK1, phosphoinositide-dependent protein kinase-1; PIP<sub>2</sub>, phosphatidylinositol 4,5-bisphosphate; PIP<sub>3</sub>, phosphatidylinositol 3,4,5-trisphosphate; PH, pleckstrin homology.

*soma cruzi* glycoprotein known as cruzipain (24) and lipopolysaccharide (253, 282). An immunomodulatory agent, glucan phosphate, has also been shown to preserve myocardium and activate AKT (252). Low dose *N,N*-dimethylsphingosine (DMS), a sphingosine kinase inhibitor, enhances epidermal growth factor receptor signaling leading to an increase in AKT activity (329). Exogenous treatment with ceramide, a sphingomyelin breakdown product, has also been shown to be cardioprotective in a manner similar to ischemic preconditioning (127). The positive inotropic ouabain, which is toxic in high doses, induces hypertrophy via AKT in low doses (424). Treatment with the NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) induces phospho-AKT along with vasoactive effects (384). VO(OPT), bis(1-oxy-2-pyridinethiolato)oxovanadium(IV), is a tyrosine phosphatase inhibitor that increases phospho-AKT related to an increase in insulin receptor phosphorylation (50, 51). Vanadyl sulfate, a VO (OPT) precursor, is currently marketed as an insulin mimetic and sports-nutritional supplement. Natural chemical compounds ingested nutritionally such as the flavonoid myricetin (20), polyphenols found in wine (36) and green tea (164) as well as phytoestrogens from soy (221) or ginsenoside Re (615), derived from ginseng root, induce AKT activation. Resveratrol, found naturally in red wine, activates AKT and is reported to be both anti-apoptotic and anti-hypertrophic in the myocardium (129, 131, 234).

Exogenous overexpression of certain enzymes also results in cardioprotection associated with an increase in phospho-AKT. Calcineurin, a calcium/calmodulin regulated phosphatase, is pro-hypertrophic but also anti-apoptotic when adenovirally or genetically overexpressed in the myocardium (139, 279). Cardiomyocytes transduced with cGMP-dependent protein kinase G show increased phospho-AKT as well as resistance to both necrosis and apoptosis (125). H11 kinase mediates hypertrophy via AKT but is reportedly toxic at high doses (149, 267). Exogenous overexpression of heme oxygenase-1 can augment activated AKT levels induced by other agents (198). The enzyme kallikrein cleaves kininogen to the protective peptide kinin. Overexpression of kallikrein increases phospho-AKT levels and is anti-apoptotic in myocardial infarction models (5, 408).

Extracellular stimuli influence AKT activation by nonparacrine mechanisms such as mechanotransduction (57) or cell-cell contact. Mechanical stress induced by regional ischemia, inflation of an intraventricular balloon, or creation of an aortocaval shunt all lead to increased AKT activation (357). A muscle specific  $\beta$ 1-integrin interacting protein named melusin appears to exert cardioprotective properties linked to AKT activation (137). Myocardial hypoxia followed by reperfusion is a powerful trigger for AKT activation (90). However, not all cells in the myocardium will respond comparably, as stimuli mediating AKT activity

**Table 2** Summary of AKT upstream inductive signaling

Category	Name	Receptor/Target	Receptor Type	Effect	Reference Nos.
Endocrine	Adrenomedullin	Calcitonin receptor-like	GPCR	Anti-apoptotic, multiple cardioprotective mechanisms	675
Endocrine	Angiotensin II	Angiotensin II receptor	GPCR	Anti-apoptotic, hypertrophic, vasoactive	128, 130, 228, 285
Endocrine	Atrial natriuretic peptide (ANP)	Natriuretic peptide receptor A/B/C	Guanylyl cyclase	Anti-apoptotic	347
Endocrine	Erythropoietin	EPO receptor	Hematopoietin receptor superfamily		359, 515, 635
Endocrine	Estrogen	Estrogen G protein-coupled receptor	GPCR	Anti-hypertrophic, anti-apoptotic	306, 532, 534
Endocrine	Ghrelin	NR	N/A	Anti-apoptotic	33
Endocrine	Growth hormone (GH)	GH receptor	RTK	Hypertrophic	432
Endocrine	Insulin	Insulin receptor, IGF1 receptor	RTK	Anti-apoptotic	12, 48, 213
Endocrine	Resistin	Unknown	N/A	Anti-apoptotic	214
Endocrine	Thyroid hormone	Thyroid receptor $\alpha$ 1	Intracellular	Hypertrophic, anti-apoptotic	350, 386, 388
Cytokine	Angiopoietin	Integrins, Tie2		Anti-hypertrophic	122
Cytokine	Cardiotrophin	gp130/LIFR		Anti-apoptotic	59, 385
Cytokine	Granulocyte colony-stimulating factor (G-CSF)	G-CSF receptor	Hematopoietin receptor superfamily	Anti-autophagic, myocardial regeneration	415, 486
Cytokine	Insulin-like growth factor I (IGF-I)	IGF1 receptor	RTK	Anti-apoptotic, hypertrophic	158, 298, 411, 672
Cytokine	Interleukin-18	IL-18 receptor	Immunoglobulin superfamily	Not antiapoptotic, hypertrophic	88, 112
Cytokine	Leukemia inhibitory factor (LIF)	LIF receptor (CD118) / gp130	RTK	Anti-apoptotic, hypertrophic	289, 502
Cytokine	Neuregulin-1	ErbB3/4	RTK	Anti-apoptotic, hypertrophic, contractility	207, 404, 631
Cytokine	Platelet-derived growth factor (PDGF)	PDGF receptor	RTK	Anti-apoptotic, hypertrophic, pro-proliferative	286, 300

Table 2—Continued

Category	Name	Receptor/Target	Receptor Type	Effect	Reference Nos.
Cytokine	Stromal cell-derived factor 1	CXCR4	GPCR	Anti-apoptotic	302, 572
Cytokine	Urocortin	CRF-R2	GPCR	Anti-apoptotic	58
Cytokine	WNT1-induced Secreted protein-1			Hypertrophic, pro-fibrotic	113
Other: peptide	Bradykinin	Via epidermal growth factor receptor	RTK	Anti-apoptotic	108, 478
Other: peptide	Endothelin (ET)	ET-(A, B1, B2)	GPCR	Anti-apoptotic, vasoactive	577
Other: peptide	Thymosin $\beta$ 4	Integrin linked kinase	Integrin linked kinase	Anti-apoptotic	54
Drug/small molecule	Acetylcholine	Muscarinic Acetylcholine receptor	GPCR	Anti-apoptotic	339, 371
Drug/small molecule	Adenosine/ Adenosine-like agonists	Adenosine A1/A3 receptor	GPCR	Anti-apoptotic, vasoactive	227
Drug/small molecule	$\beta_2$ -Adrenergic agonists, zinterol	$\beta_2$ Adrenergic receptor	GPCR	Anti-apoptotic	96
Drug/small molecule	Cannabinoids	Cannabinoid $\beta$ 1 receptor	GPCR	NR	284
Drug/small molecule	Ceramide	NR	N/A	Anti-apoptotic	127
Drug/small molecule	Cruzipain	NR	N/A	Anti-apoptotic	24
Drug/small molecule	Eplerenone	Mineralocorticoid receptor antagonist	Intracellular	NR	363
Drug/small molecule	Glucan phosphate	NR	N/A	Anti-apoptotic	252
Drug/small molecule	Isoflurane and related compounds	Via adenosine A1 receptor	GPCR	Anti-apoptotic	324, 557, 698
Drug/small molecule	Lipopolysaccharide	Toll-like receptor 4	Toll-like receptor	Anti-apoptotic	253, 282
Drug/small molecule	Morphine	Opioid receptor	GPCR	NR	243
Drug/small molecule	N,N-dimethylsphingosine (DMS)	Via epidermal growth Factor receptor	RTK	NR	329
Drug/small molecule	Quabain	Na <sup>+</sup> -K <sup>+</sup> -ATPase	Ion Pump	Hypertrophic	424
Drug/small molecule	Phenylephrine	$\alpha$ 1 Adrenergic receptor	GPCR	Hypertrophic	106
Drug/small molecule	Rosiglitazone	PPAR gamma	Intracellular	Anti-apoptotic	356, 691

Table 2—Continued

Category	Name	Receptor/Target	Receptor Type	Effect	Reference Nos.
Drug/small molecule	S-nitroso-N-acetylpenicillamine (SNAP)	NR	N/A	NR	384
Drug/small molecule	Statins	RhoA inhibition	N/A	Anti-hypertrophic, anti-apoptotic	268, 417
Drug/small molecule	VO(OPT), bis(1-oxy-2-pyridinethiolato)oxovanadium(IV)	Tyrosine phosphatase inhibitor	N/A	Anti-apoptotic	50, 51
Dietary agent	Ginsenoside	NR	NR	Anti-necrotic	615
Dietary agent	Myricetin	NR	N/A	Vasoactive	20
Dietary agent	Phytoestrogen	Estrogen receptor $\alpha$	Intracellular	Anti-hypertrophic	221
Dietary agent	Polyphenols	NR	N/A	Angiogenic, free radical scavenger	36, 164
Dietary agent	Resveratrol	Adenosine A3 receptor	GPCR	Anti-apoptotic, anti-hypertrophic	129, 131, 234
Enzyme: phosphatase	Calcineurin	N/A	N/A	Anti-apoptotic, hypertrophic	139, 279
Enzyme: kinase	cGMP-dependent protein kinase G	N/A	N/A	Anti-apoptotic, anti-necrotic	125
Enzyme: kinase, Heat shock protein	H11 kinase	N/A	N/A	Hypertrophic	149, 267
Enzyme	Heme oxygenase-1	N/A, ANG II required	N/A	Anti-apoptotic	198
Enzyme	Kallekrein-kinin	ACE/kinin B2 receptor	Transmembrane Zinc Metallopeptidase	Anti-apoptotic, anti-hypertrophic	5, 408

GPCR, G protein-coupled receptor, RTK, receptor tyrosine kinase, NR, not reported, N/A, not applicable.

(234) are likely to show context-dependent cell type differences such as those observed in cardiac fibroblasts (111). Osmotic stress can also activate stress kinases including AKT such as hyperosmolarity induced by sorbitol or mannitol (212). In congruence with AKT's role as a central mediator of growth and survival signaling, it is to be expected that the wide variety of signals described above would act as upstream inductive signals to AKT activation.

## B. Antagonists: GSK3 $\beta$ , PTEN

The dependence of AKT activity on upstream regulation by PI3K had been demonstrated in numerous studies. The production of phosphoinositides by PI3K is reversed by phosphoinositide phosphatases. Protein phosphatase and tensin homolog (PTEN) deleted on chromosome 10 possesses phosphoinositide phosphatase activity, and activation of PTEN results in inactivation of AKT. PTEN protein levels are decreased in conjunction with preconditioning concomitant with increased AKT activation, supporting reciprocity in the PTEN antagonism of AKT activity (74). PTEN also participates in regulation of hypertrophic remodeling and influences contractility via effects on PI3K signaling that lies upstream of AKT activity (119). In PTEN null hearts, there is an increased level of phospho-AKT/PKB (serine-473), and the inactivation of PTEN in cardiomyocytes results in hypertrophy. The hypertrophy found in PTEN-deficient hearts displayed features characteristic of physiological hypertrophy, such as increase in both the length and width of the myocytes, no fibrotic changes, and no decompensation into dilated cardiomyopathy. Recently, it was shown that loss of PTEN prevents the development of maladaptive ventricular remodeling with preservation of angiogenesis and metabolic gene expression in response to pressure overload (521). Consistent with the critical role for AKT in cell survival, gain of PTEN activity leads to enhanced apoptosis, and increased expression of PTEN induces an expected increase of apoptosis in neonatal cardiomyocytes (578).

GSK-3 is a serine/threonine kinase that phosphorylates and inactivates glycogen synthase, and the ability of AKT to inhibit GSK-3 $\beta$  via phosphorylation and repressive effects of GSK-3 $\beta$  upon AKT actions is a classic study in reciprocal molecular antagonism (264). GSK-3 has two mammalian isoforms: GSK-3 $\alpha$  and - $\beta$ , which are both expressed in heart. GSK-3 $\beta$  is constitutively active in unstimulated cells where it phosphorylates several targets (in addition to glycogen synthase) including cyclin D, c-Jun, NFAT proteins, and  $\beta$ -catenin leading to their inactivation and/or degradation. Phosphorylation of serine-9 residue in NH<sub>2</sub>-terminal region of GSK-3 $\beta$  by AKT inhibits GSK-3 $\beta$ , thereby leading to diverse effects including improved cell survival and hypertrophy, and improves contractile function in pressure-overloaded hearts, implying the activity of AKT as a cardioprotective mechanism (35, 261).

Direct dephosphorylation and inactivation of AKT is mediated by other phosphatases and inhibitory interactions. Another negative regulator of AKT activity in cardiomyocytes are 14-3-3 proteins and poly (ADP-ribose) polymerase 1 (PARP). 14-3-3 proteins are a family of regulatory molecules that are found ubiquitously in eukaryotes. 14-3-3 proteins inhibit cardiomyocyte hypertrophic responses, and AKT activity is also blunted by the 14-3-3 proteins that inhibit hypertrophy (418). The PARP family of enzymes has many intracellular functions, including transcriptional regulation, detection of DNA strand breaks and initiation of repair to damaged DNA. Inhibition of PARP resulted in a significant increase in phospho-AKT, and inhibition of PARP helps protect the cardiomyocyte from impaired function following ischemia (215, 370). Pleiotrophin is a developmentally regulated cytokine and AKT antagonist. Pleiotrophin antagonizes IGF-I associated Ser-473 phosphorylation of AKT/PKB, and it concomitantly decreases phosphorylation of downstream AKT targets such as BAD and GSK-3 (410). Protein-tyrosine-phosphatase-1B overexpression (PTP1B) negatively regulates insulin signaling leading to inhibition of AKT phosphorylation (175). However, the exact role of PTP1B in cardiomyocytes remains to be defined. Three less characterized pathways that alter AKT activity are TNF- $\alpha$ , MyD88, and Toll-like receptor4 (TLR4). Growth factor TNF- $\alpha$  overexpression results in inhibition of AKT that was dependent on upregulation of NF $\kappa$ B (283). Inhibition of the MyD88 pathway also protects the myocardium from ischemia reperfusion injury via activation of AKT (304) and deletion of TLR4 results in enhanced AKT-dependent cardioprotection (305). The exact role of these pathways and their contribution to altered AKT signaling in disease states also remain to be defined.

## C. Downstream Target Molecules: GSK, TORC, FOXO, BCL, BAD, etc.

The most widely studied downstream target of AKT is GSK-3, a proline-directed serine/threonine kinase that regulates a wide range of cellular processes including glycogen metabolism, gene transcription, protein translation, and cell apoptosis. GSK-3 has two isoforms in mammalian cells, GSK-3 $\alpha$  (51 kDa) and GSK-3 $\beta$  (47 kDa). AKT phosphorylates both GSK-3 $\alpha$  (Ser-21) and GSK-3 $\beta$  (Ser-9) to inhibit their activity. Overexpression of a constitutively active phosphomimetic mutant of AKT (E40K) induces cardiac hypertrophy by phosphorylation of GSK-3 $\beta$  and upregulation of GATA4 (114), although adenoviral injection (460) or transgenic overexpression (455, 584) of another constitutively active AKT, AKT-myr, enhances kinase activity without phosphorylation of GSK-3 $\beta$ . Both GSK-3 $\alpha$  and GSK-3 $\beta$  are expressed in mammalian heart and negatively regulate cardiac hypertrophy, but most studies are focused on GSK-3 $\beta$ . GSK-3 $\beta$  localizes predominantly in the cytosol but is also found in the nucleus and mitochondria. Under basal unstimulated conditions, GSK-3 is highly active and

inhibits glycogen synthesis by phosphorylation of glycogen synthase. GSK-3 negatively regulates gene transcription and protein translation by phosphorylation of a range of transcription regulators (NFAT, GATA4, myocardin, c-Myc, c-Jun,  $\beta$ -catenin) and translation initiation factor eIF2B. GSK-3 phosphorylates NFAT and promotes nuclear export (39, 647) as well as proteasomal degradation of NFAT (683). Cardiac-specific expression of GSK-3 $\beta$  attenuates pressure overload-induced hypertrophy by inhibiting the increase of nuclear NFAT (22). Cardiac transcription factor GATA4 is also exported from the nucleus after phosphorylation by GSK3 $\beta$  (490). Myocardin, another cardiac-specific transcription factor, is also phosphorylated by GSK3 $\beta$ , which reduces intrinsic myocardin transcriptional activity and related hypertrophy (30). Inhibition of PI3K/AKT signaling activates GSK-3, which accumulates in the nucleus (40), where GSK-3 phosphorylates c-Myc on Thr-58, thereby promoting its ubiquitination and degradation (241, 308). Similarly, phosphorylation of c-Jun by GSK3 resulted in binding of E3 ligase Fbw7, which targets c-Jun to proteasomal degradation (665). Inhibition of GSK-3 activity by AKT is critical to hypertrophic stimulus-induced stabilization of the transcriptional activator  $\beta$ -catenin (263). Eukaryotic initiation factor eIF2B, which regulates the initiation of mRNA translation, can be phosphorylated and inactivated by GSK-3 (666). Phosphorylation of eIF2B inhibits protein function and, in turn, accounts for the anti-hypertrophic effect of GSK-3 $\beta$  (265). The result of eliminating GSK-3 $\beta$  is hypertrophic cardiomyopathy in knock-out mice associated with increased expression of GATA4, cyclin D1, and c-Myc (353). In addition to inhibition of cardiomyocyte hypertrophy, GSK-3 $\beta$  also promotes apoptosis by the intrinsic mitochondrial pathway (464, 662) in cardiomyocytes (477). Cardiac-specific overexpression of dominant negative GSK-3 $\beta$  induces compensatory hypertrophy and inhibits apoptosis by myeloid cell leukemia-1 (291). Similarly, GSK-3 $\alpha$  is also antihypertrophic and proapoptotic, but apparently through a different mechanism, i.e., inhibition of ERK activity (687). During zebrafish cardiogenesis, the deletion of GSK-3 $\alpha$  increases cardiomyocyte apoptosis, whereas deletion of GSK-3 $\beta$  disrupts left-right asymmetry and heart positioning (400). While phosphorylation of GSK-3 $\beta$  (S9) mediates pathological hypertrophy, phosphorylation of GSK-3 $\alpha$  (S21; predominantly in nucleus) negatively regulates hypertrophy during pressure overload (453). Differential remodeling responses occur following mutation of GSK-3 $\alpha$  or GSK-3 $\beta$ , resulting from altered phosphorylation at AKT target residues of GSK-3 $\alpha$  (S21A) or GSK-3 $\beta$  (S9A) when expressed in mice. As research progresses, more differences between GSK-3 $\alpha$  and GSK-3 $\beta$  are likely to be revealed.

The Forkhead (FOXO) family of transcription factors are well-known AKT targets. FOXO factors regulate transcription of several genes possessing the 5'-TTGTTTAC-3' sequence in their promoter region (211). Cell cycle regulators

p27kip (cyclin dependent kinase inhibitor) and p130 are influenced by FOXO, along with proapoptotic molecules BIM and Fas ligand. AKT phosphorylates FOXO1, FOXO3a, and FOXO4, resulting in export from the nucleus and attenuation of FOXO-mediated apoptosis (664). Phosphorylation of FOXO factors by AKT creates docking sites for subsequent interaction with 14-3-3 proteins, leading to cytosolic sequestration as a mechanism to inhibit proapoptotic function. Nuclear targeted AKT increases cytosolic FOXO levels, potentially facilitating protection against ischemic injury in mice overexpressing nuclear AKT (76, 620).

Telomere maintenance is influenced by AKT via phosphorylation of telomere repeat binding factor 1 (TRF1) (94) and telomerase (TERT) (256). These phosphorylation events seem to have opposing effects depending on cell type. Telomeres shorten when AKT phosphorylates TRF1 in HEK293T cells (94), whereas TERT phosphorylation increases enzyme activity and has been shown to be protective in cardiac cells (513, 514). Further studies will need to elucidate the role of AKT in relation to genomic stability, specifically telomere preservation.

The BCL-2 family member BAD (BCL-xL/BCL-2 associated death promoter) contributes to cellular apoptosis by heterodimerizing with BCL-xL/BCL-2 and neutralizing their protective effect (676). AKT phosphorylation of BAD at Ser-136 disrupts the dimerization between BAD and BCL-xL (133, 144) and inhibits apoptosis (438). Phosphorylated BAD is sequestered in the cytosol through binding to 14-3-3 (686). In cultured cardiomyocytes, cardiotrophin-1 promotes survival by phosphorylation of BAD through a PI3K/AKT-dependent pathway (385). Leukemia inhibitory factor (LIF) prevents doxorubicin-induced cardiomyocyte apoptosis by PI3K-mediated phosphorylation of BAD, disrupting heterodimerization of BAD with BCL-xL (502). In adult heart, doxorubicin upregulates phosphatase 1, which dephosphorylates AKT and its downstream target S136-BAD (187). Cardiac resynchronization of dogs with dyssynchronous heart failure is accompanied by increased AKT activity, marked BAD phosphorylation, and enhanced BAD/14-3-3 interaction (87). Kallikrein gene delivery attenuates ischemia/reperfusion-induced cardiomyocyte apoptosis through increased phosphorylation of AKT and BAD (S136) (681). Thus antagonism of BAD by AKT-mediated phosphorylation plays a central role in regulating cell survival.

TOR (target of rapamycin), a serine/threonine kinase, was originally discovered by Heitman and colleagues in a genetic screen of yeast mutants whereby resistance to growth was conferred via inhibition of the immunosuppressant complex FKBP (FK506 binding protein)-rapamycin (280). The corresponding 289-kDa mammalian homolog mTOR was then identified (61, 99, 563)

and confirmed as a novel downstream target of AKT (501).

mTOR serves as a central node in multiple tissue types, including the heart, for cellular signaling particularly in terms of “sensing” environmental stimuli including, but not limited to, nutrient availability (such as insulin, glucose, and amino acids), growth factors (such as PDGF and EGF), and hypoxia (as observed within infarction after heart attack) (reviewed in Ref. 395). Upon examination of these stimuli and their effects on mTOR, researchers have elucidated a more complex signaling mechanism between AKT and mTOR. First, the tuberin (TSC2)/hamartin (TSC1) tumor suppressor protein complex was identified as a key modulator between AKT and its activation of mTOR. Upon activation of AKT, TSC2 is phosphorylated, thereby disrupting its association with TSC1. Disruption of this complex is accompanied by activation (i.e., phosphorylation) of mTOR (218, 319, 541). Further research into the TSC2/TSC1 protein complex has led to the discovery of another intermediary between AKT and mTOR: the small GTPase, Rheb (Ras homologue enriched in brain). Once the TSC2/TSC1 complex is dissociated, phosphorylated TSC2 activates the GTP form of Rheb, thereby allowing Rheb to directly bind to and activate mTOR (414, 429, 626, 693).

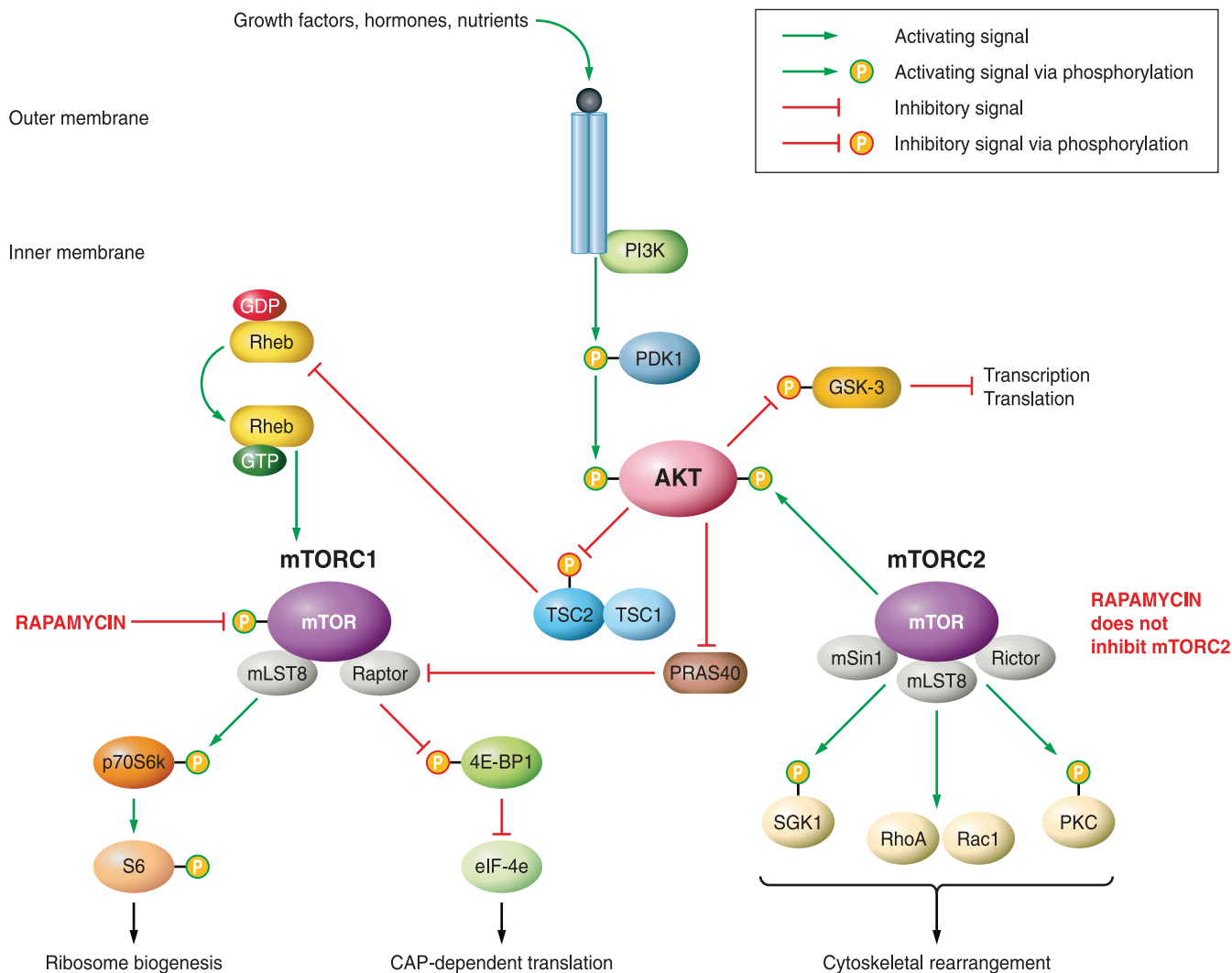
Signaling through PI3K-AKT-TSC1/2-Rheb was considered to be the main avenue through which most activating stimuli are transduced to mTOR. However, recent studies have indicated another novel mechanism by which AKT signaling bypasses the TSC2-Rheb portion to directly activate mTOR. PRAS40 (proline-rich AKT/PKB substrate of 40 kDa) was identified through coimmunoprecipitation experiments as a negative regulator of mTOR. Researchers illustrated that under basal conditions PRAS40 binds to mTOR to inactivate it, and that mTOR inactivation is relieved when insulin stimulation activates AKT to phosphorylate PRAS40, thereby initiating release from mTOR (566, 651). Furthermore, coimmunoprecipitation studies have revealed two functionally distinct mTOR complexes: mTORC1 and mTORC2 (273). mTORC1 associates with Raptor and mLST8, creating a complex that is sensitive to the mTOR inhibitor rapamycin (668). mTORC2 binds to Rictor, mSIN1, and mLST8 to form a complex that is considered rapamycin insensitive (568) unless treated chronically (569). Most of the signaling in the myocardium between AKT and mTOR has been observed through mTORC1, and this portion of the review will focus on those interactions. However, it is important to note that full activation of AKT to signal to mTORC1 is necessitated through phosphorylation via mTORC2 (FIGURE 2).

Activation of mTORC1 has been linked to numerous cancers and proliferative cell disorders including myocardial hypertrophy (reviewed in Ref. 246). Regulation of

mTORC1 via AKT is central to coordinating the regulation of two important cellular processes: 1) cell size and mass and 2) cellular proliferation/cell cycle progression. Upon activation of mTORC1, two downstream targets are dually affected with opposing end-target effects. One downstream target, p70S6k, is phosphorylated and directly activates the ribosomal protein S6, a component of the 40S ribosomal subunit. Activation of S6 ultimately leads to increased ribosomal biogenesis and activated metabolism (reviewed in Ref. 325). Decreased cell size, as observed in *Drosophila* (690) and mammalian models (472, 535, 580), has been associated with inactivating mutations in p70S6k. Another downstream target, 4E-BP1, is inactivated by phosphorylation via mTOR. 4E-BP1, when hypophosphorylated, binds to and inactivates elongation initiation factor 4E (eIF4E), thereby inhibiting CAP-dependent translation. Inactivation/phosphorylation of 4E-BP1 therefore allows for activation of protein translation and ultimately cellular proliferation (reviewed in Ref. 325). Consequently, regulation of mTOR is central to the coordinated regulation of both cellular proliferation (via p70S6k and 4E-BP1) and cell size (via p70S6k).

The role of mTOR in the myocardium, particularly with regard to cardiac hypertrophy, has attracted increasing interest within the last 10 years. Several studies have indicated a crucial role for AKT-mTORC1 signaling in the heart. Initial studies of insulin growth factor (IGF-I) overexpression in the heart proved that PI3K/AKT signaling is crucial in the development of cardiac hypertrophy (548). Research into heart-specific (under the control of the  $\alpha$ -myosin heavy chain promoter) murine models of either overexpressed (584), constitutively activated (116), or membrane localized (via myristoylation) (456) AKT further confirmed the role of PI3K/AKT in cardiac hypertrophy. However, the link between AKT/mTORC1 signaling and cardiac hypertrophy was first established by observation of AKT/mTORC1 pathway activation in cultured cardiac myocytes (512). Subsequent studies confirmed AKT signaling through mTOR produces myocardial hypertrophic growth (TABLE 3). Even now, current models of cardiac hypertrophy demonstrate increased AKT/mTORC1 signaling [i.e., hypercholesterolemia (387), spontaneous hypertension (230)].

Rapamycin has been touted in the cardiology field as an important therapeutic strategy for preventing restenosis (194). From favorable responses observed in treating patients with rapamycin-treated stents (272), several studies have validated that pharmacological inhibition of mTOR (via rapamycin and/or rapamycin analogs) reduces hypertrophic remodeling observed in cardiovascular disease (such as hypertensive-, diabetic-, or hypercholesteremic-induced cardiac hypertrophy) (73, 355, 470, 586). Preclinical trial research continues to assess feasibility of rapamycin as a treatment for cardiovascular disease.



**FIGURE 2** Pathways of AKT influencing the mTOR protein complexes. Schematic diagram representing the regulatory functions of the mTORC1 and mTORC2 complexes in relation to AKT signaling and cellular outcomes. Induction of AKT activity by extracellular signals results in the activation of mTORC1. mTORC2 activity positively regulates AKT activity. Green arrows represent positive regulation. Green arrows leading to phosphorylation represent activation via phosphorylation. Red arrows represent negative regulation. Red arrows leading to phosphorylation represent repression via phosphorylation.

Signaling through AKT inevitably leads to changes in both gene expression as well as metabolism that are inextricably linked (reviewed in Ref. 105). Intracellular NO production depends on AKT activity (143). Control of metabolic signaling is also involved, as AKT activity regulates insulin-induced regulation of 6-phosphofructo-2 kinase in the heart (150). AKT activity has been linked to antagonizing  $\beta$ 1-adrenergic receptor activity by promoting internalization (225). AKT activity phosphorylates and inhibits the action of GSK-3 $\beta$ , thereby allowing for stabilization of  $\beta$ -catenin signaling (263). Inhibition of AKT signaling blocks induction of VEGF gene expression in cardiomyocytes (277). AKT also blunts activation of AMP-activated protein kinase (AMPK)  $\alpha$  by phosphorylation (299), and AKT activation can lead to decreased AMPK activity (369). AKT also phosphorylates and activates p70S6 kinase, resulting in cardioprotection (333, 334).

#### D. Consequences for Protein Expression/Repression

Consequences of AKT activation for gene expression have been studied in transgenic mice engineered with cardiac-specific expression of myristoylated AKT resulting in a broad range of effects on genes controlling cardiomyocyte survival, metabolism, and growth (115). Cataloging these effects of altered AKT expression provides interesting insights into consequences of aberrant activity, with the caveat that the resultant listing of target genes is likely to be skewed by the nonphysiological timing, level of induction, and profound remodeling of the myocardium resulting from chronic AKT activity. Activation of cardiac AKT increases the anti-apoptotic protein FSTL1 (517) and insulin-like growth factor-binding protein-5 (IGFBP-5) and decreases PPAR $\alpha$ /PGC-1 $\alpha$  transcripts, which plays a critical



**Table 3** Cardiac hypertrophy models targeting AKT/mTOR signaling

Hypertrophy Model	Model System	Protein Studied	Effect on Myocardium	Reference Nos.
Cardiac IGF-I	Murine	AKT; p70S6k	Hypertrophy; increased cardiomyocyte proliferation	548
Whole body OE-AKT	Murine	AKT	Hypertrophy	585
Whole body AKT KO	Murine	AKT	Pathological hypertrophy; reduced body size	142
Cardiac CA-AKT	Murine	AKT; p70S6k	Hypertrophy; reduced contractility; increased cardiomyocyte size	116
Cardiac Myr-AKT	Murine	AKT; p70S6k	Hypertrophy; contractility not affected	116; 456
Cardiac DN-AKT	Murine	AKT; p70S6k	Reduced heart and cardiomyocyte size; contractility not affected	585
Whole body p70S6k KO	<i>Drosophila</i> ; murine	p70S6k	No hypertrophy; reduced body size and cell size	690; 535, 580*
Cardiac CA-GSK3 $\beta$	Murine	GSK-3 $\beta$	Hypertrophy	21
Cardiac inducible GSK3 $\beta$	Murine	GSK-3 $\beta$	Reversal of hypertrophy	564
Thyroid hormone	Rat	AKT; mTOR	Hypertrophy	387
Hypercholesterolemia	Swine	mTOR	Hypertrophy	230
Hypertension	Rat	mTOR	Hypertrophy	598

OE, overexpressed; CA, constitutively active; Myr, myristylated; DN, dominant-negative; KO, knock-out.

role in myocardial energy metabolism (115). Physiological cardiac growth, which is accompanied by increased PPAR $\alpha$ /PGC-1, is associated with increased fatty acid and oxygen consumption. Conversely, pathological hypertrophy is related to decreased PPAR $\alpha$ -PGC-1 $\alpha$  expression and a shift towards glycolysis that allows continued ATP production with less oxygen consumption (172). Activation of AKT increases sarcolemmal expression of GLUT4, leading to higher levels of glucose uptake and cardiac metabolism (460). By using an inducible AKT transgenic mouse model, Schiekofer et al. (576) showed that acute AKT1 activation (2 wk) that changes expression of 826 transcripts results in reversible hypertrophy with maintained contractility. In comparison, chronic AKT1 activation (6 wk) that changed expression of 1,611 transcripts leads to severe cardiac hypertrophy and dysfunction (576). In another report, chronic AKT activation induces dramatically larger infarcts in response to ischemia-reperfusion through feedback inhibition of PI3K activity by decreasing insulin receptor substrate-1 (IRS-1) (498). Administration of insulin (213, 334) or IGF-I (136, 203) reduces postischemic myocardial apoptotic death and infarct size by activating the PI3K/AKT signaling pathway. Loss-of-function experiments have also been utilized to study the physiological effects of AKT. Knockout of AKT1 gene results in growth retardation and increased spontaneous apoptosis in mice (92, 101). Knockout of AKT2 leads to insulin resistance (100, 222) and enhanced apoptosis in response to myocardial ischemia (140). Double knockout of AKT1/AKT2 causes severe deficiency in development of skin, bone, and skeletal muscle and mice die shortly after birth (536). Combined deletion of AKT1/AKT3 leads to embryonic lethality with severe developmental defects in the cardiovascular and nervous systems (679). The survival of single knockout mice suggests functional redundancy among the three AKT isoforms. PI3K activates AKT through phosphorylation of PIP<sub>2</sub> to form PIP<sub>3</sub>. Class IA PI3Ks (PI3K $\alpha$ ,  $\beta$ , and  $\delta$ ), which are activated by receptor tyrosine kinases (RTKs) in response to cytokines/growth factors (insulin, IGF-I, etc.), regulate physiological growth during development. In contrast, class IB PI3K (PI3K $\gamma$ ), which is activated by G protein-coupled receptor (GPCR) agonists (endothelin-1, ANG II,  $\alpha$ -AR and  $\beta$ -AR agonists) and pressure overload, leads to pathological hypertrophy (522). At basal conditions, cardiac-specific expression of constitutively active PI3K $\alpha$  results in larger hearts, while dominant-negative PI3K results in smaller hearts (583). However, mice expressing a dominant-negative PI3K (p110 $\alpha$ ) mutant display significant hypertrophy in response to pressure overload but not exercise training (473). Subsequent studies using PI3K (p110 $\alpha$ ) overexpressing transgenic mice have shown that PI3K $\alpha$  blunts cardiomyocyte hypertrophy induced by pressure overload but not exercise training (468), indicating PI3K $\alpha$  is critical for the induction of physiological cardiac growth but not pathological growth. Cardiac-specific deletion of the PI3K p85 $\alpha/\beta$  regulatory subunits attenuates AKT signaling and

exercise-induced cardiac hypertrophy (435). PI3K $\gamma$ -deficient mice exhibit less activation of AKT/ERK1/2 and attenuated hypertrophy in response to isoproterenol (520) and transverse aortic constriction (530). Consistent with this paradigm, AKT1 null mice are resistant to swimming-induced cardiac hypertrophy. Unexpectedly, when subjected to pressure overload, the AKT1 null mice develop an exacerbated form of cardiac hypertrophy (141). Based on these findings, the authors propose that AKT1 promotes physiological hypertrophy and suppresses pathological cardiac hypertrophy.

Studies with altered myocardial AKT activity reveal a cornucopia of phenotypic outcomes. PI3K inhibitors wortmannin and LY294002 attenuate the protection of insulin (213, 334), IGF-I (203), NRG-1 (207), ischemic preconditioning (270, 362, 487), postconditioning (637) against cardiomyocyte apoptosis, and ischemia-reperfusion injury by preventing AKT phosphorylation. The lipid phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10) negatively regulates the PI3K/AKT signaling pathway by dephosphorylating PIP<sub>3</sub>. Overexpression of PTEN causes cardiomyocyte apoptosis through inhibition of PI3K signaling (578). In contrast, inactivation of PTEN induces cardiomyocyte hypertrophy through PI3K $\alpha$  and decreases myocardial contractility through PI3K $\gamma$  (119, 578). Mice deficient in PTEN display basal hypertrophy and mild reduction in systolic function, yet exhibit reduced pathological hypertrophy and apoptosis with preserved left ventricular function in response to pressure overload (521). Consistently, inducible cardiac-specific deletion of PTEN activates AKT and protects the heart from ischemia/reperfusion injury (561). Activation of PI3K leads to AKT phosphorylation at Thr-308 by phosphoinositide-dependent kinase 1 (PDK1) (14) and Ser-473 by the rictor-mTOR complex (570). Cardiac-specific knockout of PDK1 abolishes the activation of AKT by insulin and results in heart failure through reduced cardiomyocyte volume (489) and increased apoptosis (320). Administration of IGF-I or deletion of PTEN increases the density of L-type Ca<sup>2+</sup> channel (LTCC) through the PI3K-AKT pathway, leading to increased Ca<sup>2+</sup> influx and cardiac contractility (613, 654). Recently, by using PDK1-deficient mice, AKT has also been shown to increase LTCC protein density and improve sarcoplasmic reticulum (SR) Ca<sup>2+</sup> handling through phosphorylation of Ca<sub>v</sub> $\beta$ 2 (82) and phospholamban (81).

Another facet of AKT activity is the potentiation and enhancement of stem cell-mediated regeneration and repair, whether by direct or indirect mechanisms (231, 234, 364, 390, 420, 446). Transplantation of mesenchymal stem cells overexpressing AKT reduces infarct size and prevents remodeling due to decreased stem cell apoptosis (420, 446). AKT increases secretion of paracrine factors (VEGF, IGF, SFRP2) (231, 233, 482) but not differentiation (509) and plays a critical role in these reports of cardioprotection. In

cardiac stem cells, overexpression of AKT promotes proliferation (244), whereas inhibition of AKT activity impairs proliferation and induces apoptosis (624). On the basis of these findings, it seems reasonable to propose that alterations of AKT activity will influence the reparative and regenerative potential of the myocardium.

#### IV. ALTERING AKT SIGNALING

Physiological regulation of AKT occurs via triggering of membrane receptors and subsequent regulation of downstream activity by phosphatases such as PTEN (401, 441) or PHLPP2 (216) which depress AKT kinase activity via dephosphorylation. The dynamic and transient nature of receptor-driven activation of kinase signaling makes determination of AKT functional effects more challenging. Therefore, overexpression systems using molecular biology tools have created a variety of altered AKT signaling constructs with activities that are heightened, impaired, or targeted to specific subcellular compartments. These tools have yielded much of the literature dedicated to AKT function in myocardial contexts with important insights into regulation of signaling and remodeling by manipulation of AKT activity in aberrant ways that may reflect pathophysiological conditions. However, it is important to remember that all such endeavors take a decidedly nonphysiological approach to examining AKT function and that understanding the normal physiological role of AKT is best served by models that mimic the consequences of AKT activity observed under physiological stimulation by the inductive signals detailed in the previous section. Thus the following molecularly engineered forms of AKT have been essential in elucidating its many functions to varying degrees.

##### A. Wild-Type AKT Expression

AKT normally exists in the cellular milieu with activity regulated by posttranslational phosphorylation. A significant effect of overexpression of wild-type AKT has not been reported in the literature. The available data indicate that AKT activity in cardiomyocytes is tightly controlled by signaling events originating at the membrane. Knock-out of AKT1 in mice results in an impaired growth phenotype (101), but cardiac-specific data have yet to be reported.

##### B. Myristoylated AKT Expression

Modification of AKT with a myristoylation moiety (myr-AKT) results in enhanced plasma membrane association that encourages proximity to the constitutively active PDK enzyme which leads to AKT phosphorylation and activity (365). Adenoviral-mediated expression of myr-AKT protects cultured neonatal cardiomyocytes from hypoxia-induced apoptosis (454). In vivo gene transfer utilizing adenoviral expression vectors of myr-AKT in the setting of acute

ischemia-reperfusion challenge results in smaller infarct size and preservation of cardiac function in rat models (460). Transgenic mice expressing cardiac-specific myr-AKT exhibit cardiomyocyte hypertrophy along with an increase in heart size, although cardiac function is preserved (455). These mice are also protected from ischemic injury and show reduced scar size after myocardial infarction (455).

### C. Dominant Negative Expression

As might be expected from the protective effects of AKT activation, the inhibition of AKT via dominant negative constructs with impaired phosphorylation sites leads to increased susceptibility to apoptotic challenge and can block the protective effects of agents such as IGF-I or neuregulin-1 that normally act to prevent apoptosis via induction of AKT activity (203, 207). To produce these phenotypes, site-specific mutagenesis has been utilized to produce nonactivated forms of AKT by replacing PDK1 phosphorylation sites threonine 308 and serine 473 with either alanine (328) or aspartate (23). In addition to becoming susceptible to apoptotic stimuli, inactive AKT promotes mitochondrial electrochemical dysfunction (391), reduces protein synthesis, impairs calcium transients (460), and results in diminished cardiomyocyte size (114). Mutations in the PH domain render the kinase unable to bind to phospholipids or proteins sequestering it away from the membrane and unable to be activated by PDK (459). These dominant negative mutations mimic phenotypes seen in AKT-specific phosphatase overexpressing transgenics including PTEN (578, 592) and PP2A (553, 636). Mutations to AKT in the ATP binding site (K179M) produced an inactive form of the kinase with some lethality 2 and 11 wk after birth (584). These kinase dead transgenics have blunted downstream target activity, but not all activity is completely attenuated consistent with the dominant negative phenotype. Morphometric and hemodynamic analysis revealed no statistical difference in hearts from these mutants versus control (584), suggesting endogenous compensatory signaling.

### D. Phosphomimetic Expression

Another modification of AKT allowing for increased activity is the substitution of charged residues at selected sites, thereby creating a phosphomimetic mutant that is purported to possess enhanced affinity for PI3K-generated phospholipids by a substitution of glutamic acid (E) for a lysine residue (K) at position 40 in the pleckstrin homology domain (43). Thus the activity of the E40K mutant is higher than that of wild-type nonphosphorylated AKT but much lower than myristoylated AKT. Cardiac-specific transgenesis with the E40K mutant leads to cardiomyocyte hypertrophy, cardiac remodeling, and increased contractility (114). Post mortem analysis revealed that transgenic mice have increased total heart mass, right and left chamber

mass, and heart weight-to-body weight ratios (114). Histochemical analysis revealed increased concentric myocyte hypertrophy without fibrosis (114). Upon further analysis increased hemodynamic function was subsequently associated with increased expression and activity of SR  $\text{Ca}^{2+}$ -ATPase 2a (SERCA2A) (361). The increased SERCA2A activity in these transgenics was attributed to the ability of AKT to directly phosphorylate phospholamban, a regulatory protein associated with SERCA at Thr-17 (81). GSK-3 $\beta$  is another downstream target hyper-phosphorylated particularly in E40K transgenic hearts and not myristoylated and wild-type mutants (114, 584). Phosphorylation of GSK-3 $\beta$  by AKT is known to inhibit the activity of GSK-3 $\beta$  and is essential for both physiological and pathological hypertrophy (163). These signals in concert contribute to the ability of these E40K transgenic hearts to withstand pathological challenge by pressure overload with reduced apoptotic cell death (84). Interestingly, mice with phosphomimetic overexpression of AKT do not have elevated levels of phosphorylated p44/42 MAPK signals commonly associated with physiological hypertrophy (104). Phosphorylation of AKT on Thr-308 and Ser-473 is essential for activation; mutations on these residues to aspartic acid imitate the negative charge of phosphorylation and produce a phosphomimetic phenotype. Mice with myocyte specific overexpression of this mutation have elevated S6 kinase activity, suggesting increased protein synthesis and hypertrophy (584). Chronic AKT activation has produced both beneficial and deleterious results (455, 633, 672). Perhaps this is due to the levels of AKT overexpression; the transgenics that survive have increased concentric hypertrophy, enhanced cardiomyocyte glucose uptake, and tend to be functionally normal (457).

### E. Nuclear Targeted Expression

The concept of nuclear AKT accumulation playing a critical role in myocardial biology was originally championed in seminal studies demonstrating nuclear localization of AKT in the myocardium (76, 611). In the first of a series of nuclear-targeted AKT-related publications, a wild-type AKT was used to maintain near-physiological levels of kinase activity with targeting mediated by a concatameric nuclear localization sequence. Nuclear accumulation of AKT produced profound anti-apoptotic activity without evidence of hypertrophic growth in either cultured cardiomyocytes or genetically engineered mice that specifically expressed nuclear targeted AKT (590). Inhibition of apoptosis met or exceeded that of myristoylated AKT, and prevention of ischemia/reperfusion damage in vivo was comparable to the potent effect of preconditioning. Striking similarities between cardiac-specific expression of nuclear-targeted AKT or IGF indicated the identification of a pivotal requirement for AKT activation, allowing for beneficial characteristics of IGF-mediated protection without maladaptive hypertrophy or undesirable paracrine-signaling side effects. Indeed, subsequent publications have demon-

strated that nuclear accumulation of AKT is actually anti-hypertrophic (641), in agreement with findings obtained with AKT knockout mice (141). Furthermore, the proliferation of myocardial stem and progenitor cell populations is enhanced by myocardial-specific nuclear AKT expression, casting new light on the implementation of AKT activity as a molecular interventional approach for treatment of cardiomyopathic damage resulting from acute injury, chronic stress, or the debilitating changes of aging (244, 616).

## V. RELATIONSHIP OF AKT TO PIM-1 KINASE

### A. PIM-1 Biology

Pro-survival and proliferative effects of AKT activity in the myocardium are well documented (203, 590, 656). However, recent evidence indicates that these actions previously ascribed to AKT are actually mediated by a downstream kinase called PIM-1, one of a three member family of serine/threonine kinases belonging to the calmodulin-dependent protein kinase (CAMK) related group (69, 295). Similar to AKT in several respects, PIM-1 is also a serine/threonine kinase originally identified as a cellular oncogene that inhibits apoptosis and promotes proliferation (29, 661). PIM-1 is expressed in various hematopoietic sites including thymus, spleen, bone marrow, and fetal liver, but can also be found in oral epithelia, prostate, hippocampus (in response to seizures), vascular smooth muscle (in response to injury), and many tumorigenic cell types (176, 344, 399, 416, 445, 474, 497, 605). In comparison, adult myocardium exhibits relatively low PIM-1 expression under normal conditions. Induction of PIM-1 is mediated through a variety of growth factors that can involve JAK/STAT pathway signaling with rapid accumulation of protein reminiscent of an early response gene (451, 533, 608). AKT signaling has also been linked to induction of PIM-1 expression resulting from prolactin treatment (372).

### B. Cross-Talk Between AKT and PIM-1

Similar to AKT, PIM-1 has many substrate targets that participate in gene transcription, cell cycle regulation, signal transduction, and antagonizing apoptosis. For example, both AKT and PIM-1 both directly phosphorylate and inactivate BAD, a proapoptotic BCL-2 family member (10, 133, 673). Additional intersections exist at targets controlling the I $\kappa$ B/NF $\kappa$ B transcription factor complex, regulation of protein synthesis via mTOR, and GSK-3 $\beta$  phosphorylation (17). Overlapping roles for AKT and PIM-1 as regulators of cellular proliferation and survival were found in studies of nontransformed hematopoietic stem cells (260). Furthermore, the pharmacological compound LY294002 previously thought to specifically inhibit PI3K and subsequent AKT activation also directly inhibits PIM-1, suggest-

ing that effects previously ascribed to blockade of PI3K/AKT need to be reinterpreted for potential consequences of concurrent PIM-1 inhibition (323). Collectively, these observations point to a close interrelationship between AKT and PIM-1 in cellular signaling. However, mechanistically there is a pivotal distinction between the two kinases: whereas AKT is activated by posttranslational phosphorylation, PIM-1 is constitutively active and therefore must be controlled by protein turnover involving regulation at transcriptional, posttranscriptional, translational, and post-translational levels. Thus, while AKT may be present but inactive, the only way to decrease PIM-1 activity is rapid turnover through proteosomal degradation (661).

### C. Implications for Myocardial Biology

Our group extended observations of PIM-1 expression to include the myocardium, where PIM-1 expression is found in cardiomyocytes of the postnatal heart and is downregulated within a few weeks after birth (492). Induction of PIM-1 occurs after pathological challenge to the adult heart, with accumulation and persistence of PIM-1 in surviving myocytes that border areas of infarction. Cardiac-specific expression of PIM-1 was highly protective in response to infarction challenge, whereas genetic deletion of PIM-1 rendered mice more susceptible to infarction damage despite significant compensatory increases in AKT expression and phosphorylation. These findings point to PIM-1 as a critical downstream participant in AKT-mediated cardioprotection, with implications for PIM-1 as a participant in survival, proliferative, and reparative processes previously associated with AKT activity. Future studies expanding on the role of myocardial PIM-1 may lead to more focused avenues for intervening in cellular processes rather than AKT, since PIM-1 activity can be directly regulated by expression level and may not have the widespread and often deleterious (114) impact of altered AKT signaling previously observed in the heart (455, 457, 498, 584, 587, 656).

## VI. MITOCHONDRIA

### A. Mitochondrial Integrity and Survival Kinases

The critical role of mitochondria as arbiters of cell survival is widely recognized and well documented in the myocardial context (4, 126, 145, 271, 317, 326, 466, 495, 500, 688). Since mitochondria act as integrators of multiple cellular conditions reflecting physiological and genomic stresses, it is reasonable to expect that kinase signaling mechanisms influencing cell survival impinge either directly or indirectly on mitochondrial integrity. Indeed, a cornucopia of studies have documented the influence of each major kinase signaling pathway on mitochondrial activity including PKA, PKC, ERK, JNK, p38, and AKT (296). AKT pro-

tective signaling has been shown to act on many levels of mitochondrial function. Outer mitochondrial membrane integrity, predominately controlled by BCL-2 family proteins, is both directly and indirectly affected by AKT activity. Moreover, certain cardioprotective effects of AKT have been suggested to depend on translocation from the cytosol to mitochondria (7), where it inhibits opening of the permeability transition pore (mPTP) to maintain mitochondrial integrity (135, 326, 336). Connections between insulin, IGF, and cardiotrophin on AKT signaling point to the interplay of AKT with mitochondria (391). As the role of AKT in protection of mitochondrial integrity has recently been reviewed in detail (525), this section summarizes basic principles of intrinsic cell death and focuses on recent advances regarding hexokinases in the context of the myocardium.

## B. Intrinsic Apoptotic Cascades

Mitochondrial-dependent apoptosis, also referred to as programmed cell death, is activated in response to a variety of extracellular or intracellular insults initiating a multiplicity of downstream cascades. Intrinsic apoptotic events predominantly center on mitochondrial integrity which acts as a cumulative breaking point upon which apoptosis hinges (259, 463, 546). Therefore, mitochondria act as cellular “executioners” by releasing pro-apoptotic molecules normally held within the intermembrane space such as cytochrome *c*, apoptosis inducing factor (AIF), Smac/Diablo, HtrA2/Omi, and endonuclease G (545). Once mitochondrial membrane integrity is breached, these activators of apoptotic cascades lead to cell death via multiple independent mechanisms. Thus a critical facet of inhibiting apoptosis is prevention of mitochondrial membrane permeabilization.

The stability of mitochondrial membranes is largely dictated by the BCL-2 proteins, a large family of both pro- and anti-apoptotic members that exist in a dynamic balance. Interaction of AKT with two of these BCL-2 members BAD and BAX has been the focus of considerable attention. BAD promotes apoptosis by forming heterodimers with anti-apoptotic BCL-2 or BCL-xL proteins, thereby inhibiting their protective effects. In comparison, BAX undergoes a conformational shift that allows for its insertion into mitochondrial membranes and oligomerization with cytochrome *c* to promote membrane permeabilization (251). AKT antagonizes pro-apoptotic actions of these BCL-2 family members by kinase activity, phosphorylating both BAD (Ser-136) (133) as well as BAX (Ser-184) (671). Phosphorylation dissociates BAD from complexes with BCL-2/BCL-xL proteins and promotes association with 14-3-3 to sequester BAD in the cytosol, thereby negating interference of BAD with protective signaling (301). Recently, new lines of evidence have indicated that BAD may play a more direct role in cell homeostasis in addition to its well-known action

of inhibiting anti-apoptotic BCL-2 proteins. One such target is mPTP. Dephosphorylation of BAD by PP2A or by inhibition of PKA and PKC sensitizes PTP to  $\text{Ca}^{2+}$  by ceramide, an effect that is independent of BAX and BAK (560). As ceramide treatment increased BAD/BCL-xL interaction, PTP sensitization may be due in part to BCL-xL activation, although its putative activity is drawn into question as BAX and BAK are thought to be targets of BCL-xL. Recent evidence also suggests that phosphorylation of the BH3 domain of BAD, responsible for the anti-apoptotic activity of BAD, has an additional role in glucokinase activity and glucose-stimulated insulin secretion (124), again promoting the observation that metabolic and survival/apoptotic signaling not only interact but share many common substrates. Although these additional roles have not been shown in the heart, glucose metabolism in the heart is a likely intersection of the known roles of AKT, BAD phosphorylation, and hexokinase regulation. This interaction might be especially prevalent as ischemic myocytes are believed to upregulate glycolysis in response to increased ADP/ATP ratios and that further stimulation of glycolysis protects against myocyte failure during ischemia and reperfusion (331, 332).

The consequence of BAX phosphorylation by AKT is to promote heterodimerization with BCL-xL or MCL-1 (a BCL-2 related protein), thereby sequestering BAX away from mitochondrial membranes (219). Alternatively, AKT may directly interfere with molecules that promote conformational change of BAX, such as BID or BIF-1. Through modulation of cytosolic BCL-2 family members via phosphorylation, AKT regulates the initiation of mitochondrial membrane permeabilization that leads to apoptosis. Multiple studies have ferreted out the relationship between AKT activation, BCL-2 family member regulation, and inhibition of cardiomyopathic damage (334, 345, 385, 502, 528, 643). Although inhibition of BAX translocation via phosphorylation by AKT has not been shown in the heart, BAX  $-/-$  mice are protected against ischemia-reperfusion injury (292). AKT has also been suggested to suppress activities of pro-apoptotic molecules released from compromised mitochondria such as AIF and HtrA2/Omi (93, 678), but these observations in nonmyocardial contexts will require further studies to validate their role in the heart. Additionally, mitochondrial integrity is impacted by effects of AKT activity via altered gene transcription of forkhead family members, MDM2, NF $\kappa$ B, CREB, and YAP (38, 63, 123, 165). Thus AKT controls a multifaceted array of downstream mediators that are directly or indirectly responsible for regulating mitochondrial integrity.

## C. Hexokinase: Targets of AKT in Mitochondria

The intertwined relationship linking AKT to preservation of mitochondria creates the mechanistic basis for a sensing mechanism to regulate cellular energy metabolism and sur-

vival. Consider that most stimuli for cellular growth and proliferation operate via AKT-associated signaling to promote energy utilization derived from mitochondrial function. As such, preservation of mitochondrial integrity is a synergistic consequence of AKT function that enhances growth and survival processes via kinase activity that consumes ATP to phosphorylate target molecules. Availability of energy substrates is critical for growth and survival, and AKT also has a dependence on glucose to antagonize apoptotic signaling. Current speculation posits that metabolic functions of AKT preceded and eventually evolved into additional roles in preservation of cell survival as well, with glucose-dependent antiapoptotic signaling of AKT interfacing through phosphorylation of hexokinases to protect mitochondria.

Fueling this model of AKT/mitochondrial symbiosis, a strong correlation also exists for preservation of mitochondrial integrity by AKT action to promote localization and stabilization of mitochondrial hexokinase on the outer membrane. Hexokinases (HKs) regulate glucose uptake and metabolism chiefly by phosphorylating free intracellular glucose. The product of this reaction, glucose-6-phosphate, cannot pass back through the plasma membrane and thus maintains a positive glucose gradient from the bloodstream (601). Under basal conditions, hexokinase activity is regulated positively by insulin, and negatively by its product. There are four isoforms of hexokinase, with the heart expressing mainly variants I and II (602). Many tissues, including the heart, respond to metabolic stress such as hypoxia or ischemia by upregulating hexokinase activity in an effort to maintain critical ATP levels. Studies over the last 15 years have shown that HK plays a protective role at least in part through glycolytic signaling. Recently, the association of HK with mitochondria has been implicated as an important mechanism of HK-mediated protection. HKs I and II contain NH<sub>2</sub>-terminal mitochondrial-binding motifs, and overexpression of truncated forms resulted in reduce protection against H<sub>2</sub>O<sub>2</sub>-induced MPT pore opening in neonatal cardiomyocytes (614). Treatment with volatile anesthetics or ischemic preconditioning (IPC), both known to be cardioprotective following ischemia/reperfusion insult, promote HK association with the mitochondria, corroborating in vitro data (248, 700). Mitochondrial HK may also support mitochondrial membrane integrity by occupying VDAC binding sites, making them unavailable to BAX/BAK recruitment or by reducing oxidative stress (554, 567). Recent studies of AKT in HK-dependent survival have revealed the interdependence of these pathways in protecting the heart. Several lines of evidence support HK as the facilitator of AKT-mediated protective signaling: 1) ectopic expression of HK mimics the effects of AKT activation to inhibit apoptosis (443), 2) both AKT and HK require glucose for antiapoptotic activity (544), 3) targeted disruption of HK interactions with mitochondria impairs the protective actions of AKT (442), 4) association of HK with mitochondria is impaired in AKT-deficient cells following

growth factor stimulation, and 5) glucose deprivation reduces HK association with mitochondria (436). More recently it has been shown that AKT can act directly on mitochondria to phosphorylate HK-II, resulting in protection from oxidant or calcium-stimulated permeability transition pore opening in cardiomyocytes (484). In conclusion, although we are at the tip of the proverbial iceberg with regard to assessing this new facet of AKT-mediated signaling in the myocardium, the increasingly apparent codependence of AKT and mitochondria for mutual functional activity points to an inexorably linked partnership through HK that may be the evolutionary interface designed to balance energy conditions, cell metabolism, growth, and survival under stress.

## VII. CONTRACTILITY AND CALCIUM SIGNALING

### A. Functional Effects

Gain- and loss-of-function studies comprehensively characterized AKT1 as a regulator of contractility and calcium cycling in cardiac myocytes, and enhanced contractility can be observed in a variety of settings associated with enhanced AKT activity both in vitro and in vivo. Conversely, impairment of this signaling pathway is an important determinant of cardiac malfunction. For example, cardiac specific overexpression of IGF-I receptor and knockout of insulin receptor resulted in enhanced and reduced cardiac contractility, respectively (523, 654). Of clinical importance and in accordance with results from animal models, it is known that IGF-I treatment of the failing human heart leads to enhanced contractility (156, 157).

For the physiological stimulus of endurance exercise (e.g., swim training), hemodynamic stress results in adaptive myocyte growth with preserved contractile function (physiological hypertrophy). In contrast, pathological stimuli such as pressure overload lead to hypertrophy, which often progresses to heart failure (278). The differences between physiological or pathological cardiac hypertrophy are most likely due to differences in proximal signaling pathways. Whereas activation of G protein-coupled receptors is necessary to induce pathological hypertrophy, insulin or IGF-I coupled to the PI3K/AKT1 pathway has been associated with physiological growth of the heart (588). In line with this, studies have shown that inhibition of PI3K or genetic ablation of AKT1 prevents exercise-induced hypertrophy (140, 141, 473), which indicates that PI3K/AKT is required for compensatory growth in the heart. In line with this, increased inotropism, lusitropism, and improved calcium dynamics were observed following physiological adaptive hypertrophy following exercise training and in experimental models of elevated activity of the IGF-PI3K-AKT signaling cascade (103, 349, 699).

Multiple studies have proven that the molecular mechanisms of AKT regarding the enhanced contractility are conveyed by direct consequences for calcium handling by either directly or indirectly modifying the function of proteins responsible for calcium cycling (103, 114, 119, 361, 396, 558, 590, 613).

The process of excitation-contraction coupling (ECC) in skeletal and cardiac muscle cells requires membrane depolarization. After membrane depolarization  $\text{Ca}^{2+}$  influx is activated via voltage-gated L-type  $\text{Ca}^{2+}$  channels into the cytosol of both skeletal muscle cells and cardiac myocytes (46). This rise in cytoplasmic  $\text{Ca}^{2+}$  concentration leads to  $\text{Ca}^{2+}$  release from the SR ( $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release; CICR) by activation of ryanodine receptors (RyR). After  $\text{Ca}^{2+}$  release of the RyR,  $\text{Ca}^{2+}$  molecules subsequently bind to the contractile proteins such as troponin c, which causes contraction of the myocytes. Thereafter,  $\text{Ca}^{2+}$  is cleared from the cytosol by reuptake of  $\text{Ca}^{2+}$  into the SR by the action of a SERCA. As discussed in detail below, both plasma membrane and SR calcium fluxes required for contraction are regulated by AKT activity.

## B. Contractile Effects

Associations between AKT1 activity and calcium handling proteins were initially observed in experimental models of cardiomyopathy wherein decreased AKT1 activation was concurrent with diminished SERCA, NCX, and PLB phosphorylation (167). Conversely, in transgenic mice with cardiac specific overexpression of AKT, it was shown that the amplitude of  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ) was enhanced in AKT myocytes compared with that in wild-type myocytes, which may be at least in part responsible for the enhanced cellular  $\text{Ca}^{2+}$  transients (114, 361). Second, an increased protein expression of the SERCA could be identified as another molecular mechanism in transgenic mice expressing cardiac-specific constitutively active AKT. Adenoviral gene transfer of the transgene into rat myocardium (81, 103) recapitulates this phenotype. Recently, another study showed that activated AKT phosphorylates PLN at Thr-17, providing a new mechanism whereby the preferential translocation of AKT to the SR is responsible for enhancement of contractility without stimulation of hypertrophy (81).

Similarly, mice created with cardiac-specific expression of nuclear-targeted AKT also showed enhanced contractility and supraphysiological ventricular dynamics, but the molecular mechanisms responsible for the increased cardiac performance were distinctly different and were related to increased loading of the SR due to increased phosphorylation of phospholamban (Ser-16 PLB) (558). In addition, it was shown that phosphatase PP1, which dephosphorylates PLB and thereby inhibits SERCA, is downregulated in TG myocytes, providing an additional pathway for increased contractility.

Taken together, these studies indicate that cardiac specific AKT1, whether constitutively active or nuclear targeted, improves contractility through elevated  $\text{Ca}^{2+}$  handling via increases in  $I_{\text{Ca,L}}$  amplitudes or increased SERCA activity.

## VIII. ANGIOGENESIS

AKT/PKB is a pivotal regulatory kinase with various roles in growth, metabolism, and survival (80, 115, 244, 493, 590). More specifically, roles for AKT in cardiac growth as well as cardioprotection after pathological injury have been extensively documented (15, 115, 232, 244, 335, 420, 446, 462, 493, 558, 562, 590, 591, 593, 641). To date, a variety of studies attribute short-term AKT activation to the profound protective effects seen in posts ischemic injury models by which AKT increases cell cycling and inhibits apoptosis (364, 590, 641). AKT activation has also been demonstrated to stimulate neoangiogenesis and vasculogenesis (3, 84, 439, 491, 575, 579, 588, 621), in part accounting for the dramatic improvements and survival of myocardial tissue.

During embryonic development as well as after ischemic injury, vascular endothelial growth factor (VEGF) is secreted to initiate blood vessel formation (619). VEGF expression activates AKT through initiation of phosphorylation (2). Particularly in the heart, secretion of VEGF results in an increase in AKT phosphorylation and subsequent activation of the downstream target eNOS (154, 208). The production of NO has numerous protective effects on the vasculature including vasodilation and inhibition of intimal formation within damaged vessels (155, 519). Studies have shown deletion of eNOS and reduction of NO results in increased damage after ischemic injury. Interestingly, in transgenic mice deficient for eNOS, female mice have reduced intimal formation and better recovery after ischemic insult attributed to the AKT activator estrogen. Additional studies supporting a role for AKT in angiogenesis are demonstrated in animal models that specifically overexpress AKT in the endothelial cell lineage. Activation of AKT in endothelial cells promotes cardiac angiogenesis, increases NO production, decreases neointima formation, and results in attenuated lesion formation during ischemic injury (2, 3, 84, 154, 204, 491, 519, 575). Inhibition of PI3K or use of dominant negative AKT results in profound angiogenic and vascular defects including decreased capillarization and arteriogenesis, decreased eNOS phosphorylation, decreased endothelial cell proliferation, and reduction of NO production (439). Further studies demonstrate that AKT knockout mice possess leaky vasculature as well as impaired mobilization of endothelial progenitor cells in response to VEGF stimulation (91).

Potential of angiogenesis by AKT is increasingly apparent, particularly in the heart after ischemic injury. Animal models detailing effects of cardiac specific overexpression

of AKT indicate a decrease in infarct size with an accompanying increase in vessel and capillary density. Cardiac specific overexpression of AKT also induces a potent release of cytokines, many of which have specific roles in mediating the growth and induction of vasculogenesis. A recent molecular profiling study revealed that in hearts of mice with conditionally activated AKT, a 33% increase in vascular density was observed along with increased secretion of angiogenic paracrine factors: VEGF receptor 2, neuropillin, and connective tissue growth factor (Ctgf) compared with nontransgenic control hearts (575). Additionally, similar studies indicated AKT activation led to the release of follistatin-1 (517). Taken together, these data implicate a substantial and potentially clinically relevant role for AKT in the regulation of angiogenesis.

To date, both stem and progenitor cell types have been used to treat pathological injury after ischemic injury. Thus far, pro-angiogenic molecules have been used to modify various types of stem and progenitor cells in attempts to mitigate damage after ischemic injury. Delivery of mesenchymal stem cells modified with AKT and ANG II (591) as well as embryonic stem cells modified with VEGF (669) to areas of ischemic damage have resulted in increased AKT phosphorylation and increased angiogenesis in the surrounding tissues. These types of therapies have short-term success in attenuating damage by potentiating angiogenesis through AKT activation. However, long-term AKT overexpression has also been demonstrated to have detrimental effects, including abnormal vascular remodeling and lethal vascular defects (91, 539). While hope exists for using AKT as a novel therapeutic target to induce angiogenesis and reduce ischemic injury, a thorough understanding of effects with regard to timing and expression level is critical before implementation can be expected in the clinical arena.

## IX. RELATIONSHIPS WITH MicroRNA

The link between AKT activity and hypertrophic remodeling is indisputable, as is the recent incorporation of microRNAs (miRNA) as critical regulators of cardiac hypertrophy and failure (37, 79, 95, 448, 573, 625, 627, 649, 650). These small noncoding RNA molecules regulate gene expression and control cell growth, differentiation, and apoptosis. As these processes are central to cardiac biology, it is inevitable that miRNAs are either influenced by or participate in cardiac remodeling and pathogenesis, as borne out by studies in both experimental models and human heart samples (TABLE 4). Constitutive activation of AKT by cardiac-specific transgenesis leads to downregulation of miRNA-133 and miRNA-1, similar to changes observed following experimentally induced pressure overload or physiological hypertrophy from exercise (79). Cardiac remodeling can be stimulated or inhibited by manipulating miRNA activity. For example, in cultured cardiomyocytes, overexpression of miRNA-1 leads to inhibition of hypertrophy (573),

whereas forced expression of several stress-induced miRNAs results in hypertrophic enlargement (649). Correlations of these changes have yet to be mapped to changes in AKT expression or activity level. However, it is reasonable to speculate that the miRNAs that regulate remodeling, cell survival, and proliferation will also influence AKT, and these relationships are likely to exist in reciprocal fashion, with AKT activity influencing expression of miRNAs.

With regard to specific miRNA actions in the myocardial context, examples abound in the published literature. miR-133 has been shown to be downregulated in a cardiac hypertrophy mouse model, and overexpression of miR-133 leads to an inhibition of protein synthesis and downregulation of AKT-dependent genes. miR-133 targets the small GTPases Cdc42 and RhoA, which are implicated in cardiac hypertrophy (79). miR-126 is highly expressed in murine heart and lungs (266), and recently, miR-126 was shown to modulate VEGF-induced ERK and AKT pathways during neovascularization. Blocking miR-126 by antisense RNA in HUVECs reduces the phosphorylation of AKT and ERK activation upon VEGF stimulation. miR-126 was found to inhibit the p85 $\beta$  regulatory subunit of PI3K (PIK3R2) and Sprouty-related EVH1 domain-containing protein 1 (SPRED1) (195). These proteins negatively regulate AKT downstream of the PI3K and MAPK pathways. Hence, by negatively regulating suppressors of the AKT/ERK pathway, miR-126 acts as a positive regulator of VEGF signaling (195). A retrospective study identifies miRs differentially expressed in the heart during ischemic reperfusion and myocardial infarction (314). miR-21 was found to be highly expressed in cardiomyoblast, specifically in the infarcted zone at 7 days post myocardial infarction (MI). miR-21 targets and downregulates the AKT suppressor PTEN. miR-21 suppression of PTEN activates AKT and increases MMP-2 expression, which is implicated in cardiac remodeling post-MI (559). miR-21 also regulates Sprouty homolog 1 (Spry1), which downregulates the ERK/MAPK pathway, thereby modulating cardiac dysfunction after pressure-induced hypertrophy and MI (628). Another study demonstrated the therapeutic potential of miR-21 by reducing infarct size (680). miR-210 is upregulated in the heart during development and when the heart begins to fail. Ischemic preconditioning is known to be protective and has been shown to induce phosphorylation and activation of AKT and ERK proteins, followed by nuclear translocation of hypoxia inducible factor alpha (HIF $\alpha$ ). In response to AKT and ERK activation, miR-210 is expressed and thought to contribute to cardiomyocyte survival. The cytoprotective mechanism of ischemic preconditioning has been attributed to miR-210 suppression of caspase-8-associated protein 2 (CASP8AP2), but a direct correlation between miR-210, CASP8AP2, and AKT needs to be established (358).

Of the many miRs differentially expressed during heart diseases, some of them regulate AKT as shown in cancer stud-



**Table 4** Examples of microRNAs altered in myocardial diseases

<b>MicroRNAs Altered in Heart Diseases: (315)</b>	<b>Function</b>	<b>MicroRNAs Altered in Heart Diseases:(315)</b>	<b>Function</b>
Let7b	Developmental timing (343)	miR100	Stem cell differentiation (623), $\beta$ -adrenergic signaling (609)
Let7c	Developmental timing (377)	miR101	Epigenetic regulation (77, 653)
Let7e	Developmental timing (660)	miR103	Mesenchymal stem cell signaling (425)
mir1	Anti-hypertrophic (508)	miR106a	Cell cycle arrest and senescence (255, 405)
miR10a	Smooth muscle cell differentiation (642)	miR125b	Inhibitor of endothelin-1 (537)
miR15b	Pro-apoptotic, reduces cellular ATP (506)	miR126	Angiogenesis (55, 196, 341, 505)
miR17-5p	Both anti- and pro-proliferative (452, 674)	miR140	Reduced AKT and ERK activation (angiogenesis) (322)
miR19a, b	Target PTEN, anti-aging (255, 538)	miR145	Vascular smooth muscle cell differentiation (55, 193)
miR20a, b	Pro-proliferative, anti-aging (255, 510)	miR181a	p27 repression (121)
miR23a, b	Early hypertrophic growth, regulate metabolism (72, 421, 658)	miR195	Hypertrophic signaling (72, 649)
miR24	Cell cycle arrest (394)	miR199a	Cardiomyocyte size maintenance (600)
miR26b	Cell proliferation (504)	miR214	Cell survival (677)
miR27a, b	Pro-apoptotic, pro-differentiation (360, 689)	miR222	Neovascularization (148, 374, 375, 540, 645)
miR30	Myocardial matrix remodeling (170)	miR320	Neovascularization (552, 660)
miR93	Target VEGF (427)		

ies. miR-214 targets the AKT/PTEN pathway in ovarian cancer (677), and miR-126 reduces p85 $\beta$  of PI3K and phospho-AKT levels in colon cancer (247). miR-216a and -217 directly inhibit the PTEN and enhance the AKT activation by TGF- $\beta$  signaling (346). miR-216 induced AKT activation led to glomerular mesangial cell survival and hypertrophy in diabetic nephropathy (346). Overexpression of miR-330 in prostate cancer cell line PC3 reduced the phosphorylation of AKT by targeting the E2F1 transcription factor. Overexpression of miR-330 induced apoptosis of PC3 cells through downregulation of AKT and activation of apoptotic factors like BAD and caspase-9 (402). Although it seems various miRs interact with AKT in cancer lines, these studies need to be examined in the context of myocardium.

## X. GENDER DIFFERENCES

Differences in cardiac phenotypes between the sexes have been observed using surgically or genetically engineered experimental animal models (166). Estrogenic stimulation promotes AKT activation. Over the last several years, a number of studies have identified a link between estrogen, AKT, and cardiac remodeling or protection from failure (32, 76, 86, 152, 209, 274, 306, 367, 532, 571, 611, 638). Studies in mouse models have demonstrated the ability of estrogen to attenuate cardiac remodeling in response to pressure overload (648), and subsequent studies extended this idea to include the protective effects of estrogen following MI as well as deleterious effects of testosterone (83). Additional studies reinforced this hormone-linked impact upon cardiac remodeling in response to pathological challenge (9, 28, 70, 166, 217, 534, 594, 655), whereas other studies established correlations with estrogenic stimulation from dietary sources (220, 281, 288, 327, 574, 632) or documented gender-specific distinctions in cardiac remodeling (32, 52, 152, 166, 306, 367, 403, 494, 655). Hypertrophic remodeling dependent on AKT is influenced by p38 MAPK activity in vivo in a gender-dependent fashion, perhaps because inhibition of p38 signaling leads to enhanced estrogen-induced activation of AKT (423). The connection between estrogenic stimulation, gender, and AKT activation in the myocardium was identified by our group in studies of mouse models that documented the nuclear accumulation of AKT in response to estradiol or phytoestrogen treatment, as well as establishing differences in basal levels between males and females (76, 611). Subsequent studies documented the participation of the PI3K/AKT signaling axis in cardioprotective effects mediated by estrogenic treatment (32, 532). Since estrogen promotes nuclear accumulation of AKT (76) that is both anti-apoptotic (590) as well as antihypertrophic (640), it is reasonable to conclude that AKT activation plays a critical role in estrogen-mediated cardioprotective effects. The relevance for these connections as they relate to issues of women's health and postmenopausal hormone replacement therapy continues to be an area of active research and debate (312, 531, 551, 607).

Estrogen activation of AKT is known to influence events such as metabolism, cell cycle, and cell survival (76). Several murine models allude to the beneficial effects of estrogen and active AKT after pathological injury such as ischemia/reperfusion (I/R) injury (32). In age-matched rats, gender disparity was evident in response to I/R in vivo, where females showed an increased propensity to activate AKT and its downstream effector PKC- $\epsilon$  (32). Phenotypically female rats exhibited reduced infarct size and increased postrecovery left ventricular function after I/R injury compared with males in vivo (32). This was corroborated with the use of ovariectomized female rats and estrogen replacement by administration of 17 $\beta$ -estradiol. The proposed mechanism, which increased female resiliency to heart injury in this study, correlates with upregulated p-AKT in the nucleus (31, 76, 611). Furthermore, AKT is essential for activation of PKCs; PKC- $\epsilon$  in this system was shown to inhibit apoptosis in adverse cardiac events, and both AKT and PKC- $\epsilon$  were highly upregulated in female rats compared with males, indicative of cardioprotection (32). I/R injury is also detrimental to contractility, affecting intracellular Ca<sup>2+</sup> loading in isolated cardiomyocytes (86). Female derived cardiomyocytes exhibit less SR Ca<sup>2+</sup> loading compared with males, where altered calcium handling often leads to pro-apoptotic cascades (86). AKT downstream of PI3K has been described to influence cellular function and contractility, yet the mechanisms are still unclear.

Chronic AKT activation and localization to the nucleus is well known in the heart mediated by estrogen, but mechanistically the subcellular localization of active AKT is still being characterized in relation to sex hormones. Chronic activation of AKT and its localization in the nucleus has various physiological effects for the cell. AKT is a well-known antagonist of pro-apoptotic pathways and promotes proliferation in the cell (31). Studies that focus on the anti-apoptotic role of AKT show that with administration of estrogen, there would not only be a consistent increase in p-AKT levels in the nucleus, but an AKT-dependent inactivation and removal of the pro-apoptotic protein forkhead from the nucleus to the cytoplasm where it will be degraded (76, 611). In addition, a breast cancer model shows that women with functional estrogen receptors (ER $\alpha$ ) have more active AKT in the nucleus of their cells, leading to increased cell survival and progression of the disease (31). This ER $\alpha$ -regulated response was found to be dependent on TCL1 family members that are known to regulate the nuclear localization of AKT in different cell types (31), therefore regulating apoptotic signals.

In vitro studies have shown that 17 $\beta$ -estradiol treatments of cardiomyocytes decrease chemical-induced apoptosis. In addition, activation of AKT affects metabolic features of the cell particularly by increasing cardiac glycogen synthesis by phosphorylation and inhibition of GSK-3, which deactivates its activity (476, 657). Increase in glycogen synthesis

is beneficial in the myocardial setting because it not only increases the cells resistance to ischemic events by allowing beneficial sensitization and prolonged activity during anaerobic respiration events (657). In the effect that  $17\beta$ -estradiol is introduced to cardiomyocytes, there appears to be a substantial increase in glycogen synthesis in cardiomyocytes indicative of AKT activation (476, 657).

Sex hormones such as estrogen have been studied as potential mediators of the inflammatory response, especially in incidences of I/R in various organs (223, 526). Estrogen in particular has been shown to influence the migration and activation of leukocytes that often progress to inflammation and vessel occlusion during pathology (526). For example, estrogen administration reduced the incidence of atherosclerotic plaques, which is often worsened by chronic inflammation leading to irreversible vasoconstriction (41). Circulating cytokines after a critical inflammatory event are reduced after administration of  $17\beta$ -estradiol in burn victims, which correlated with increased p-ERK and p-AKT levels and lower apoptotic incidences (223). Sexual dimorphism becomes apparent during various ischemic events where organ remodeling facilitated by reperfusion through the vasculature is inherently "gender biased." In premenopausal woman, estrogen acts as an upstream regulator of eNOS, which by a PI3K-dependent pathway activates AKT to become phosphorylated. Activation of eNOS allows for release of NO, which has been described to facilitate vasodilation, maintain vascular tone, reduce inflammation, and support AKT dependent anti-apoptotic events via BCL-2 (275, 397, 480, 606). Contrasting evidence shows that in the renal system, when testosterone was administered to female mice there was an apparent increase in damage to the kidney assessed by inflammation and functional vasculature discrepancies in the organ (526). This deterioration of function in the kidney to eNOS and AKT was attributed to downregulation and alterations in MAPK-mediated protective pathways (526).

The sexual dichotomy of cardiovascular diseases is difficult to ignore. Various studies mentioned here are referenced to describe the apparent effects of sex hormones on pro-survival pathways most notably estrogen activation of PI3K/AKT. Ongoing studies that correlate the susceptibility of disease and morbidity of the sexes are not only limited to the heart but have reached out to studying patterns of sex hormone regulation of regeneration in the central nervous system or in easing systemic inflammation (223, 526). This study may give insight to certain physiological phenomena as to why men have higher incidences of cardiovascular diseases and decreased survival after onset of pathological insult compared with females. Similarly, women who are pregnant and have higher circulating androgens run the risk of pregnancy-induced hypertension leading to systemic organ damage (229, 330). Overall, the discrepancy between the sexes seems rooted at least in part with estrogen as a

mechanism for activating survival kinases such as AKT to blunt cardiovascular diseases and progression of systemic diseases.

## XI. EFFECTS OF CARDIOMYOPATHIC INJURY

### A. Ischemia, Reperfusion, and Pre/Postconditioning

The "reperfusion injury signaling kinase" (RISK) pathway is an initial line of defense for cardiomyocytes attempting to stave off the damaging consequences of reperfusion (419). Reperfusion activates AKT that leads to stimulation of mTOR signaling and subsequent protein synthesis (120). The PI3K-AKT and MEK1-ERK1/2 pathways cross-talk to each other and act in concert to mediate protection (269–271). The effect of AKT activity in prevention of reperfusion damage is likely connected to protection of mitochondrial integrity (258). Simulated ischemia using cultured cardiomyocytes reveals increased AKT activation, but differential timing for increased phosphorylation of the key S473 versus T308 residues (180). Human fetal cardiomyocytes are also highly resistant to hypoxic stress that may be mediated, in part, by heightened AKT activation (109). Overexpression of constitutively activated AKT protected cardiomyocytes from apoptosis in response to ischemia-reperfusion injury in vivo (203).

Different treatments before (preconditioning) or immediately with the onset of reperfusion (postconditioning) have a powerful protective effect on the myocardium to subsequent infarction challenge by activating a variety of survival pathways including AKT (118, 362, 366, 444, 637). Preconditioning the myocardium by brief alternating cycles of ischemia and reperfusion leads to reduction in infarct size that is dependent on 3' phosphoinositide-dependent kinase-1 (PDK1) (65), which presumably leads to downstream activation of AKT.

Alternative to ischemic conditioning, pharmacological conditioning would likely be more relevant to the clinical setting, e.g., ANG II mediates cardioprotective effects by enhancing reperfusion-initiated AKT phosphorylation (42). Preconditioning can be influenced by pathways that impinge on AKT-mediated effects, as shown in experiments using FRZ/sFRP-1, a secreted antagonist of the WNT/FRIZZLED pathway that inhibits phosphorylation of GSK-3 $\beta$  (34) or the transcription factor Ref-1 (249). Pharmacological preconditioning agents such as acetylcholine, bradykinin, the synthetic  $\delta$ -opioid agonist DADLE, and the anti-ischemic metabolite drug Trimetazidine increase AKT phosphorylation (108, 199, 383). Analogously, a novel member of the calcitonin/calcitonin gene-related peptide family named intermedin, the volatile anesthetic isoflurane,

or phosphodiesterase inhibitors olprinone or tadalafil reduce myocardial damage by AKT activation in the myocardial I/R model (6, 461, 599). Administration of insulin in reperfusion enhances AKT-mediated cardioprotection and leads to cross-talk between AKT and JNK pathways (422). Furthermore, different ligands of PPAR, such as the glucose-sensitizing drug Rosiglitazone or WY-14643, reduced infarct size in an AKT-dependent manner (68, 356, 692). Similarly, activation of a membrane-bound estrogen receptor or inhibition of the non-long terminal repeat retrotransposon long interspersed nuclear element 1 (LINE-1, L1) in the ischemic heart increases AKT expression and phosphorylation and functional recovery following reperfusion (151, 433). Besides steroids, peptido-hormones including adiponectin, adrenomedullin, or growth hormone releasing hormone reduce myocardial injury in an AKT-dependent manner (235, 240, 634). Stimulation of GPCRs, e.g., by SDF-1 $\alpha$ , also mediates AKT phosphorylation and prevents myocardial cell death (572). In contrast, hypercholesterolemia augmented myocardial necrosis in a pig I/R model correlating with reduced AKT phosphorylation (518).

Chemical agents are another approach to achieve preconditioning. AKT activation prompted by either carbon monoxide exposure or xenon inhalation prior to I/R challenge preconditions against injury (202, 481). Preconditioning with cobalt chloride, a hypoxia mimetic agent, or hydrogen sulfide can effectively confer cardioprotective effects prior to deep hypothermic circulatory arrest (352, 479). Preconditioning mediated by hydrogen sulfide also indicates AKT as a contributor to cardioprotection (303).

Postconditioning stimulation is more complex and AKT activity apparently depends on the duration of ischemia. Analogous to preconditioning, ischemic postconditioning is based on repeated cycles of intermittent ischemia, although treatment is applied with the onset of reperfusion following the index ischemic event. Application of ischemic postconditioning at late time points after index ischemia (more than 45 min) results in AKT activation and protective effects on cardiac injury that is attenuated by PI3K inhibition. In contrast, postconditioning performed early after index ischemia had no functional protective effect consistent with lack of AKT activation (447). Ischemic postconditioning resulting in AKT activation is initiated via JAK-STAT3 upstream of the RISK pathway (238) that can be stimulated by TNF- $\alpha$  (389). Conversely, downstream of the RISK pathway, protective effects involve alterations to GSK-3 $\beta$  and mitochondrial permeability transition (192). As an alternative approach to ischemic postconditioning, pharmacological postconditioning involves application of active components with the onset of reperfusion. As reported for preconditioning, isoflurane induces AKT activity during the process of postconditioning (97, 191, 192). Protective effects could also be achieved by postconditioning with levosimendane,

sphingosine-1-phosphate receptor agonists like FTY720, the peptido-hormone apelin, or the phytoestrogen genistein in a AKT-dependent manner (293, 294, 597, 632). Treatment with the antidiabetic drugs glimepiride (a sulfonyl urea) or metformin (a biguanide) at the onset of reperfusion limited myocardial injury in perfused Langendorff rabbit hearts, correlating with AKT phosphorylation that was antagonized by PI3K inhibitors (49, 507). Also, treatment with exenatide (a glucagon like peptide-1 agonist) limits myocardial injury with concomitant AKT phosphorylation (630). RISK survival signaling also appears augmented by administration of statins during reperfusion (174).

## B. Heart Failure, Pressure, or Volume Overload

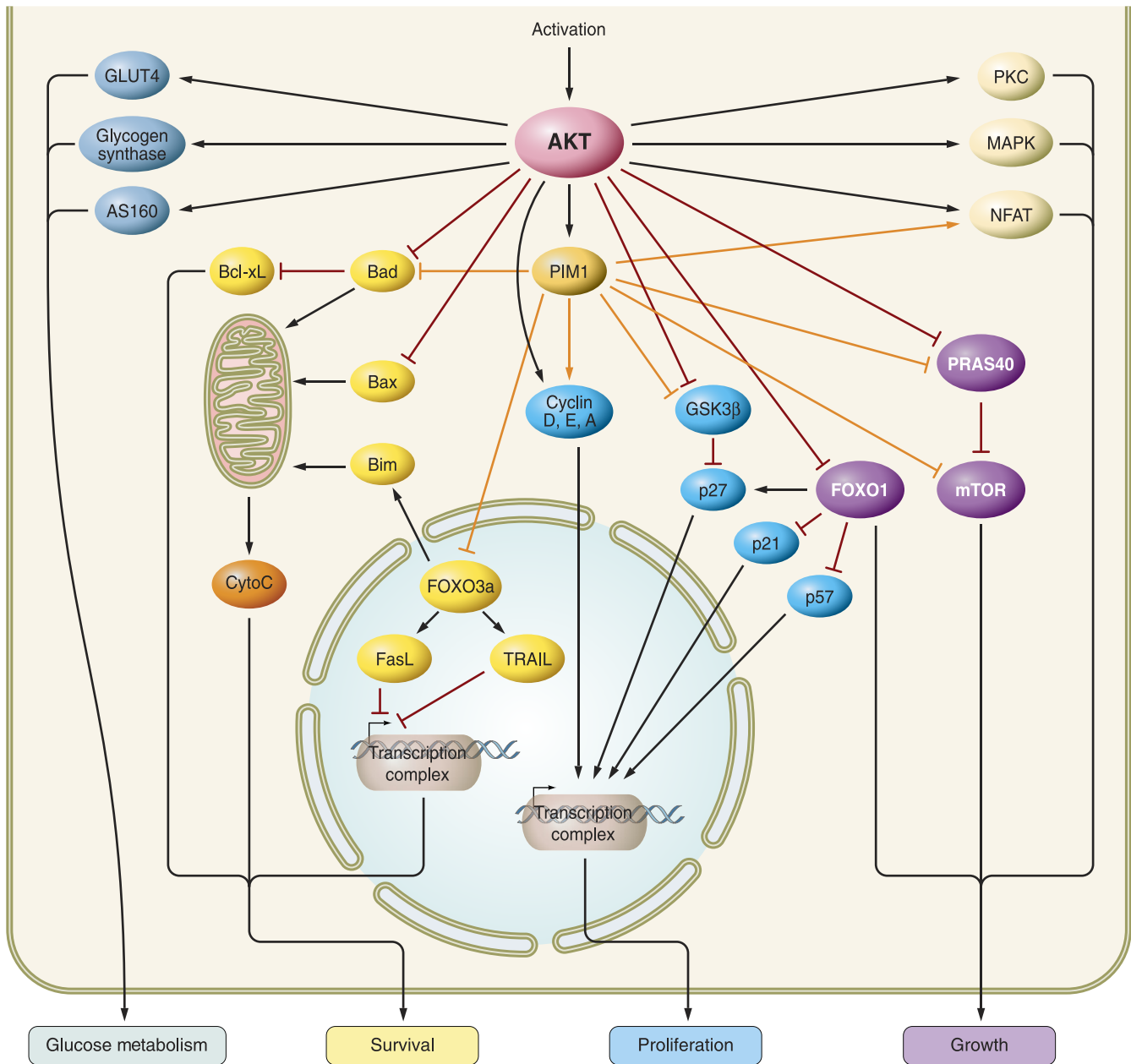
Heart failure is the common final destination of different pathological conditions such as ischemia, pressure, or volume overload. Initially, pressure-challenged hearts undergo hypertrophy, which can be beneficial by compensation in the early phase of remodeling in the stressed heart. However, ongoing hypertrophy and remodeling result secondarily in diastolic dysfunction and consequently heart failure. For this response several signaling and transcription pathways are activated including (but by no means limited to) NFAT, ERK1/2, and PI3K/AKT (66, 67, 455, 488, 618). Mechanical overload stimulates AKT activation, possibly through or in conjunction with induction of focal adhesion kinase signaling (200). AKT activation takes place after pressure as well as volume overload in rabbit models with different time courses for each (485). Heart failure resulting from volume overload is characterized by early increases in AKT activity (160) followed by decreased phosphorylation of AKT that may contribute to decompensation (147). Transgenic animals expressing cardiac-specific constitutively active AKT show a spectrum of phenotypes from cardiac hypertrophy with preserved systolic function and cardioprotection to massive cardiac dilatation and sudden death (455). In the analysis of the human specimen, AKT activation can be found in failing hearts, whereas in hypertrophic samples no AKT activation may be found (262). Depression of the gp130 survival pathway and associated depression of AKT activity are linked to human heart failure (236, 262). Chronic increases in LIF found in failing hearts may promote inhibition of AKT phosphorylation, thereby exacerbating deterioration of cardiac function (197, 236). Heart failure due to infarction damage can be blunted by postoperative treatment with G-CSF that correlates with increased VEGF and AKT activity in the ischemic region (321, 415). Under conditions of hypertrophic remodeling, dysregulation of AKT activity leads to increased susceptibility to I/R injury due to increased GSK-3 $\beta$  activity and concomitant alterations in glycolysis (35). AKT phosphorylation is downregulated in hearts of spontaneously hypertensive rats (SHR) (380) but can be normalized by exercise (393). In Dahl salt-sensitive rats with heart failure,

treatment with the aldosterone inhibitor eplerenone induced AKT activation correlating with improved cardiac function (363).

Diabetic cardiomyopathy is associated with impaired activation of AKT in streptozotocin-treated rats (250, 398) as well as diabetic rats stimulated by cardioprotective opioid treatment (243), although AKT phosphorylation may initially be increased in early stages of diabetic cardiomyopathy (437). Altered AKT phosphorylation occurs in the Zucker-diabetic-fatty (ZDF) rat model (392) and can be normalized with exercise (393). Altered AKT signaling is

also present in the mdx mouse model of dystrophin-deficient cardiomyopathy (354).

Collectively, the picture of AKT activity in the pathologically challenged heart is predictably complex, more so because there are also differences in AKT isoforms and the roles they play in prompting physiological versus pathological remodeling. However, in the end-stage failing heart where normal signal transduction has given way to dysregulated and desperate compensatory activity, the role of AKT is probably less about a particular phenotypic outcome and more indicative of the overall decline in coordinated signal



**FIGURE 3** Schematic overview of selected AKT targets, many of which are highlighted in this review. Cellular signaling around AKT and AKT substrates regulates major cellular processes in the myocardium. Activated AKT increases protein translation, cellular growth, metabolism, and cell cycle activity through regulation of downstream mediators.

transduction that depends heavily on cross-talk through AKT.

## XII. CONCLUSION: AKT AS A NODAL REGULATORY KINASE IN THE MYOCARDIUM

Despite ongoing frustration in moving toward a clinically relevant outcome for heart failure, research into the relationships between signal transduction and molecular interventional strategies to deal with cardiomyopathic disease maintain their relentless pace. The logic is pure and simple: signaling molecules regulate the biological processes of cells, so a focused and regulated approach to the right target will have the desired outcome of increasing salutary effects or blunting maladaptive consequences. But the devil is in the details, as evidenced by this review of the multifaceted nature of AKT signaling. Since most critical modulators of cell biology are present and maintained in homeostasis with concurrent environmental conditions, any targeted alteration of molecular signaling is likely to produce shrapnel effects that will compromise other aspects of cell adaptation to stress. Of course, this does not mean that the wealth of understanding related to signal transduction and myocardial biology is irrelevant to clinical treatment. On the contrary, this review shows that manipulation of AKT-dependent signaling mechanisms has the power to control a multitude of important aspects of myocardial biological processes. The limitation lies in our technical capabilities. Despite our best efforts, to date we still lack the ability to influence AKT signaling with the multifaceted and nuanced networking that typifies normal cell biology, and AKT in particular (FIGURE 3). As we delve deeper into mechanisms that regulate AKT, we will undoubtedly discover even more ways to direct, influence, and control the outcome of activation, localization, and target substrate phosphorylation. AKT is a critical nodal kinase in the cell that integrates a host of ambient information into powerful phenotypic responses. The challenge ahead is to harness this powerful signaling molecule and take advantage of the staggering spectrum of intracellular processes under the regulation of AKT.

## ACKNOWLEDGMENTS

All members of the Sussman lab contributed equally to this work.

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## GRANTS

M. A. Sussman is supported by National Heart, Lung, and Blood Institute Grants 1R21HL102714-01, 2 R01

HL067245, IR37 HL091102-01, P01HL085577-05, RC1HL100891-02, R21 HL102613-01, and 1 R21 HL104544-01. K. Fischer and N. Gude are Fellows of the Rees-Stealy Research Foundation and the San Diego State University Heart Institute. C.T. Cottage is supported by the Rees-Stealy Research Foundation, the San Diego ARCS Foundation, American Heart Association Predoctoral Fellowship 10PRE3060046, and an Inamori Foundation Fellowship. M. Völkers and M. H. Konstandin are supported by DFG Grants MV 1659 1/1 and KO 3900/1-1.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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