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PKB/Akt signaling in cardiac development and disease

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1. ABSTRACT

In mammals, there are three Akt/PKB (protein kinase B) isoforms termed Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ (hereafter referred to as Akt) that are encoded by three distinct genes localized on different chromosomes. Although the three Akt proteins share high homology and display similar domain structures, mouse genetic studies have demonstrated that they play over-lapping but also differential roles in development and physiology. In this review, we summarize recent advances in understanding the roles of Akt signaling in heart development and disease, together with discussion on Akt signaling connection to key signaling pathways in early cardiac specification. The pioneering work on Akt's function in cardiomyocytes performed by Kenneth Walsh's group, was first reported in the new millennium (1) and thus, it is now the right time to look back at some of the discoveries of Akt's role in cardiac biology over the past decade.

2. INTRODUCTION

The role of Akt in cardiac system was first revealed by the pioneering study performed by Walsh and colleagues which documented Akt's function to promote cardiomyocyte survival *in vivo* (1). Shortly after, several groups reported almost simultaneously, cardiac hypertrophy and failure in transgenic mice with Akt over-expression in cardiomyocytes (2-6). Afterwards, Akt's involvement in embryonic development has been unveiled along with the generation and characterization of Akt individual knockout mice and subsequent compound Akt deletion mice (7-15). We first reported a heart developmental defect in *Akt1/3* double knockout mice, which is in consistency with the earlier work performed in Dario Alessi's group showing severe cardiac phenotype in PDK (phosphoinositide-dependent kinase 1) germ-line deletion mice (15, 16). In the past decade, the function of Akt in heart hypertrophy and cardiac protection has been

extensively investigated while Akt's role in heart development is much less studied.

3. Akt SIGNALING IN HEART DEVELOPMENT AND DISEASE

3.1. Akt signaling in heart development

In 2002, Dario Alessi's group reported developmental defects in multiple tissues of germ-line *PDK1* deletion mice including the no-heart or heart tube phenotype (16). As Akt is one of the main downstream targets of PDK1, their results suggest Akt's involvement in cardiac development (17-20). Following this study, we deleted *PDK1* in whole mouse embryo via EIIa-Cre mediated excision (Cre expression is driven by EIIa *cis*-elements that are ubiquitously active). Similar to the observation of Alessi's group, we found that these *PDK1*-deficient embryos were severely growth-retarded and died at around E9.5. However, the heart tube could be discerned although it was poorly developed (unpublished data). The reason for the discrepancy between these studies may be due to dissection of embryos because an obvious peel-off of heart tube could be seen in the results reported by Alessi's group (16). Nonetheless, the defective heart tube development indicates PDK1's essential function in heart development probably through downstream Akt signaling.

The direct evidence of Akt's involvement in heart development came from our study of *Akt1/3* double knockout mice (15). We observed atrial septal defect (ASD) and thin myocardium in $Akt1^{-/-}3^{+/-}$ mice (15). In a following study, we found that majority of *Akt1*-deficient mice displayed congenital heart defects (CHDs) in a C57/B6 genetic background (unpublished data). Previously, Birnbaum and colleagues reported early neonatal lethality in *Akt1*-deficient mice without known reasons (21). Our results suggest that CHDs might be the cause for mortality of these mice. Observation by DeBosch and Muslin indicates that Akt1 is required for physiological cardiac growth by studying the viable *Akt1* knockout mice (22). Currently, we are investigating the underlying mechanisms for Akt1's function in heart development.

PTEN (phosphatase and tensin homolog) suppresses the PDK1-Akt signaling (23). Germ-line deletion of PTEN causes early embryonic lethality by E9.5 and endothelial-specific inactivation of PTEN delays embryonic mortality until E11.5 (23, 24). Myocardial development is impaired in endothelial-specific PTEN deletion mice indicating an essential role of *Pten* in heart development (24). These studies suggest that hyper-activation of Akt signaling may be deleterious to embryogenesis including cardiac development. However, work performed in Penninger's group points out that in adult mice, *Pten* loss in cardiomyocytes is protective against pathological hypertrophy (25).

Taken together, these reports revealed the pivotal role of PDK1-Akt signaling in heart development.

3.2. Akt signaling in cardiac remodeling and disease

Cardiac remodeling/plasticity occurs in response to two main types of stimuli: normal physiological demands and pathological insults (26, 27). Physiological demands, such as exercise or postnatal heart growth, promote cardiomyoctye growth and induce cardiac hypertrophy, thus improving heart contractility and function (28). Pathological insults, including sustained neurohumoral activation and hypertension as well as myocardial injury, result in deterioration of cardiac remodeling, a primary degenerative disease of myocardium traditionally classified into hypertrophic or dilated cardiomyopathy (HCM or DCM) (28, 29).

Akt signaling in cardiac remodeling has been extensively investigated through studying transgenic mice over-expressing constitutively active Akt (myr-Akt or Akt1T308DS473D) in cardiomyocytes (expression is driven by alpha-myosin heavy chain (aMHC) promoter) (2-6, 28-32). A number of groups have generated and characterized these Akt1 or Akt3 gain-of-function murine and their results have shown that Akt signaling controls heart size (hypertrophy). In these studies, active Akt signaling-induced cardiac hypertrophy was associated with an increase in cardiomyocyte size even through divergent phenotypes are documented. More intriguingly, Walsh's group generated a unique murine model with Akt1 activation in cardiomyocytes in an inducible manner by use of tet-on/off system (31). This study has demonstrated that cardiac hypertrophy and angiogenesis are coordinately regulated during physiological (adaptive) cardiac growth and disruption of this balance caused pathological cardiac remodeling and heart failure. During physiological (adaptive) cardiac remodeling induced by short-term Akt activation, coronary angiogenesis was enhanced along with preserved contractile function, whereas sustained (long-term) Akt activation impaired coronary angiogenesis followed by cardiomyopathy and reduced contractility (31). This study also provided insight into understanding the interplay between cardiomycytes and endothelia cells in cardiac remodeling. Through co-culture system, Walsh and colleagues demonstrated that VEGF and angiopoitin 2 (Ang-2) secreted by cardiomyocytes promote endothelial proliferation and coronary angiogenesis while endothelial cells improve cardiomyocyte growth and contractile function via an unknown mechanism (possibly through production of FGFs that function in cardiomyocyte (33).

Mechanistically, these mouse model studies revealed that Akt signaling promotes cardiac hypertrophy at least in part, through activation of β1-adrenergic and mTOR-S6K signaling pathway (2, 6, 31), and suppression of GSK3b- and FOXO-dependent atrophy programs (34, 35). In a following study of cardiac Akt transgenic mouse model, Condorelli and colleagues observed decreased expression of both miR-133 and miR-1 in the left ventricle (36). This study suggests that Akt signaling regulatory microRNAs are involved in cardiac remodeling.

Recent studies performed by us and Issei Komuro's group on cardiomyocyte-specific *PDK1* deletion mice not only confirmed the results from Dario Alessi's

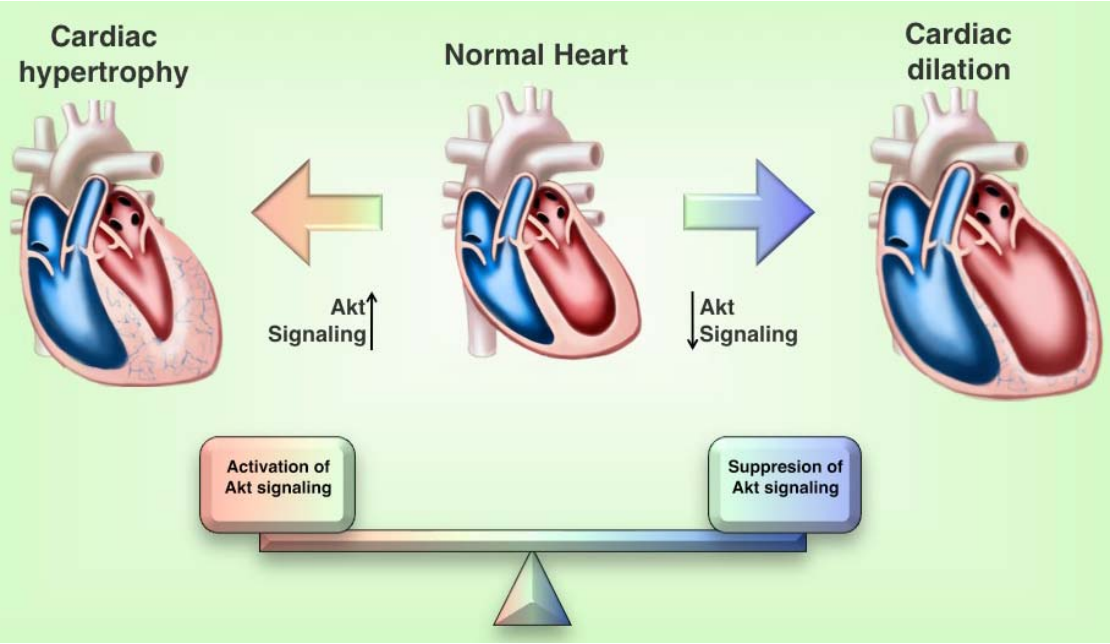

Figure 1. Akt signaling homeostasis is critical for normal heart function and its disruption regulates the switch between HCM and DCM. The balance between activation and suppression of Akt signaling in heart is finely tuned. Disruption of this balance brings about deleterious consequence of either cardiac hypertrophy/HCM or dilation/DCM. Abbrieviations: HCM: hypertrophic cardiomyopathy; DCM: dilated cardiomyopathy.

group but also pinpoint the importance of Akt signaling homeostasis in maintenance of cardiac function (37, 38). Previously, Alessi's group deleted *PDK1* in both skeletal and cardiac myocytes via MCK-Cre mediated excision and found post-natal mortality and heart failure at approximately two months (37). Recently, using a similar but different Cre line (aMHC-Cre and tomoxifen-inducible), Komuro's group inactivated PDK1 in cardiomyocytes and observed heart failure resulting from dilated cardiomyopathy (DCM) (38). Results from our study are in consistency with theirs (unpublished data). Accordingly, inactivation of PDK1 caused reduced Akt T308 phosphorylation and activity in the heart of these mice. A recent study of IGF1 receptor (IGF1R)/Insulin receptor (IR) compound deletion mice also showed that these mice developed DCM and reduced Akt phosphorylation (39).

Interestingly, it has been reported that transgenic mice over-expressing Akt E40K mutant show hypertrophy but not heart failure phenotypes, and even prevent pressure overload-induced heart failure (40). Both myr-Akt and Akt E40K mutant (mutation in the PH domain) have high affinity to lipids and are attached to membrane for activation. The discrepancy between phenotypes observed in mice with cardiomyocyte-specific myr-Akt and Akt E40K over-expression may be due to kinase activation levels because in the original paper, Vogt and colleagues revealed that Akt E40K shows a much lower activity compared to myr-Akt without known mechanism (41).

Collectively, these studies indicate that fine regulation of Akt signaling is critical for normal heart function. Disruption of Akt signaling homeostasis (either enhancement or reduction) would give rise to cardiac remodeling that, in a long run, lead to cardiomyopathy and heart failure. Meanwhile, these studies also suggest that modulation of Akt signaling could regulate the switch between HCM and DCM (Figure 1)

3.3. Akt signaling in cardiac protection

As mentioned above, the pioneering work to demonstrate Akt's function in cardiac biology was performed with a myocardial infarction (MI) model, which showed Akt's cardiac protection against ischemia ten years ago (1). Soon after, two studies revealed the upstream ligand and downstream effector of Akt signaling in cardiac protection, respectively (39, 42). It was shown that insulin administration conferred cardiac protection against ischemia through activation of Akt and subsequent phosphorylation of eNOS.

All three Akt genes were expressed in heart (15). Although Akt2-deficient mice displayed normal cardiac growth responses to provocative stimulation, these mice were found to be sensitive to myocardial infarction induced by ligation of the left coronary artery (43). This study implicates Akt2 in regulating cardiomyocyte metabolism and survival.

Furthermore, work performed in Sussman's laboratory has nicely demonstrated that nuclear Akt has an ability to antagonize cardiomyocyte hypertrophy (44). Thus, growing evidence has suggested that not only duration, but also levels of kinase activity and its cellular location play a significant role in cardiac protection

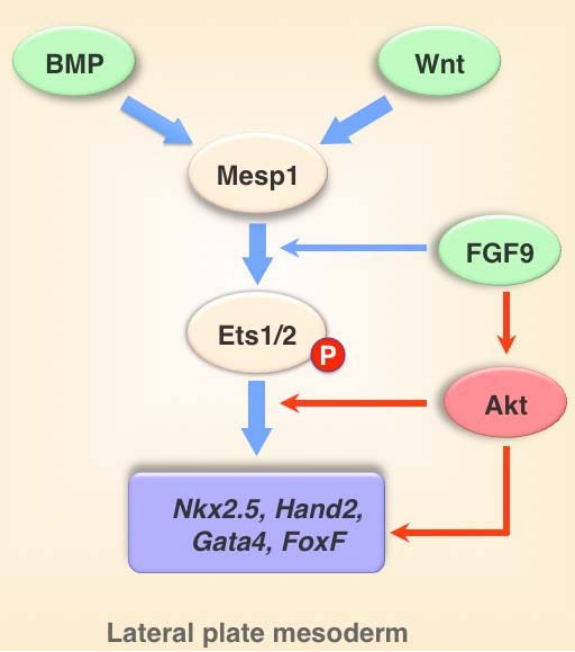

Figure 2. Potential role of Akt signaling in early cardiac specification and cardiac field induction. The key regulatory molecules and signaling pathways are summarized and modified from study of Ciona cardiac development (60, 61). Abbreviations: PKB: protein kinase B; BMP: bone morphogenetic protein; FGF: fibroblast growth factors; Ets1: E-twenty six 1; Mesp1: mesoderm posterior 1; Nkx2.5: NK transcription factor related, locus 5; Hand2: heart and neural crest derivatives 2; Gata4: zinc finger-containing transcription factor Gata-4; FoxF: forkhead box F.

Rapamycin-coated stent has been applied into patients with coronary thrombosis to reduce smooth muscle cell proliferation (45). Mechanistically, rapamycin inhibits mTOR (mammalian target of rapamycin) and hence its substrate of S6K (ribosome S6 kinase). As long-term treatment of rapamycin can induce Akt activation which in turn, promotes cell proliferation (46), the anti-thrombosis effects of the rapamycin-coated stent need to be studied cautiously.

Akt's cardiac protection has been applied to therapeutic experiment. Transplantation of rat mesenchymal stem cells over-expressing active Akt into ischemic rat myocardium inhibited cardiac remodeling and reduced fibrotic area along with improved cardiac function (47).

4. CONCLUSION AND PERSPECTIVE

A decade of study on Akt signaling in cardiac biology led to a belief that it plays a pivotal role in cardiac development, function, remodeling and protection. While the relationship between Akt signaling and cardiac remodeling and protection has been well established, how this signaling is involved in cardiac development is greatly neglected. The redundancy among Akt isoforms and early embryonic lethality of Akt double knockout mice may obstacle the investigation in this aspect. Nonetheless, generation of Akt floxed mice will facilitate the study of Akt signaling in cardiac development.

Heart is the first organ to form and function during embryogenesis and heart development is a complicated but precisely orchestrated process (28, 48-50). Four stages cover heart development: specification and induction of cardiac progenitor cells, heart tube formation, cardiac looping, and chamber growth and maturation (28, 50). Multiple signaling molecules are involved in cardiac development during each stage. Commitment to the first stage, specification and induction of cardiac cell, is the result of inductive signals from endoderm and ectoderm, which include bone morphogenetic proteins (BMPs), basic fibroblast growth factors (bFGFs) and the Wnt proteins (50) (Figure 2). Drosophila has been studied extensively as a good model animal for heart development. However, it is increasingly realized that Ciona is a simple but more appropriate model animal than fly to study cardiac development because the regulatory mechanisms are found well conserved between Ciona and mammals (51, 52). For a long time, Nkx2.5 (NK transcription factor related, locus 5) has been regarded as the earliest transcription factor expressed in cardiac tissue. Recently, three papers using *in vitro* ES cell differentiation system have demonstrated that Mesp1 (mesoderm posterior 1) is the master gene for cardiac specification and induction because Nkx2.5 expression is controlled by Mesp1 (53-55). While Mesp1 is not found in fly, it indeed exists in Ciona and functions as in mammals. Elucidation of signaling pathways guiding heart development in Ciona will greatly help understand the developmental processes in mammals (51, 52) (Figure 2).

Akt signaling could function downstream of FGFs in early cardiac specification and cardiac field induction (Figure 2). In the future, it will be of importance to study early cardiac development in Akt trio knockout mice (cardiac-specific deletion of Akt1/2/3). As Akt protein are phosphorylated by two upstream kinase and complex of PDK1 and mTORC2, cardiac-specific inactivation of PDK1 and mTORC2 may also address this question (52, 56).

Aberrant Akt signaling in heart seems to regulate the switch between HCM and DCM. The downstream effectors of Akt signaling involved in pathological remodeling need to be identified. Enhanced Akt signaling could be both blessing and curse. On one hand, increased Akt activity renders cardiac protection against myocardial infarction. On the other hand, sustained Akt activation causes hypertrophy and heart failure. How to modulate Akt activity according to disease-specific context is a big issue for future cardiac repair therapy. Preliminary results from our studies suggest that moderately enhanced Akt signaling in heart is not deleterious as transgenic mice with Akt-T308DS473D mutant expression (~2 folds as much as in wild-type) in heart live normally (unpublished data). Inhibition of heart failure resulting from hypertrophy by Akt E40K may support this point of view that mild enhancement of Akt activity is beneficial to heart function

(40). On the other hand, sustained Akt activity in nucleus may also protect heart function. In the future, it will be important to determine the threshold of aberrant Akt activation and how to maintain Akt activity in nucleus.

There is evidence that Akt signaling is involved in human cardiac diseases. For example, we found that PDK1 levels are reduced in human failing heart with DCM which is in consistency with mouse study (38) (unpublished data).

Along with the era of iPS, it could be possible to control Akt signaling activity through genome engineering to improve cardiac function in patients with HCM, DCM or MI. Recent studies suggest that inhibition of autophagy may be a novel method for therapy of heart disease (57, 58). The PI3K/Akt/mTOR signaling pathway is known to suppress autophagy in response to insulin-like and other growth factor signals (59, 60). Thus, Akt signaling could be a good target for cardiac disease treatment in the future.

5. ACKNOWLEDGEMENTS

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Abbreviations: PKB: protein kinase B; ASD: atrial septal defect; CHDs: congenital heart defects; PDK1: pyruvate dehydrogenase kinase, isozyme 1; HCM: hypertrophic cardiomyopathy; DCM dilated cardiomyopathy; Ang-2: angiopoitin 2; VEGF: vascular endothelial growth factor; FGFs: fibroblast growth factors; mTOR: mammalian target of rapamycin; S6K: S6 kinase; GSK3β: glycogen synthase kinase-3β; FOXO: forkhead box O; MCK-Cre: muscle creatine kinase-Cre; PTEN: phosphatase and tensin homolog; IGF1R: insulin-like growth factor 1 receptor; IR: Insulin receptor; MI: myocardial infarction; eNOS: endothelial nitric oxide synthase; iPS: induced pluripotent stem cell; PI3K: phosphoinositide 3-kinase; BMP: bone morphogenetic protein; FGF, fibroblast growth factors; bFGFs: basic fibroblast growth factors; Ets1: E-twenty six 1; Mesp1: mesoderm posterior 1; Nkx2.5: NK transcription factor related, locus 5; Hand2: heart and neural crest derivatives 2; Gata4: zinc finger-containing transcription factor Gata-4; FoxF: forkhead box F.

Key Words: PKB/Akt, Heart, Development, Hypertrophy, Cardiomyopathy, Review

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