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Role of diacylglycerol kinase in cellular regulatory processes:
A new regulator for cardiomyocyte hypertrophy

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Abstract

Diacylglycerol kinase (DGK) phosphorylates and converts diacylglycerol (DAG) to phosphatidic acid. DGK regulates cellular DAG levels and attenuates DAG signaling. The ten mammalian DGK isoforms have been identified to date. In cardiac myocytes, DGK α , ϵ , and ζ are expressed, and DGK ζ is the predominant isoform. DGK ζ inhibits protein kinase C activation and subsequent hypertrophic programs in response to endothelin-1 in neonatal rat cardiomyocytes. DGK ζ blocks cardiac hypertrophy induced by G protein-coupled receptor agonists and pressure-overload *in vivo*. DGK ζ attenuates ventricular remodeling and improves survival after myocardial infarction. These data provide a novel insight for subcellular mechanisms of cardiac hypertrophy and heart failure, and DGK ζ may be a new therapeutic target to prevent cardiac hypertrophy and progression to heart failure.

Key Words: diacylglycerol kinase, signal transduction, hypertrophy, heart failure, protein kinase C

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Figure Legend

Abbreviations

ANF, atrial natriuretic factor

DAG, diacylglycerol

DGK, diacylglycerol kinase

DGK ζ -TG, transgenic mouse with cardiac specific expression of DGK ζ

ERK, extracellular signal regulated kinase

ET-1, endothelin-1

GPCR, G protein coupled receptor

MHC, myosin heavy chain

PKC, protein kinase C

TAC, transverse aortic constriction

TGF, transforming growth factor

WT, wild type littermate mouse

1. Introduction

Phosphoinositide turnover is one of the key events to transducer signals of development, differentiation, and mitogenesis in response to a variety of extracellular stimuli (Cocco et al., 2004; Gonzales & Anderson, 2006). Multiple lines of experimental and clinical evidence have suggested the importance of the G α q-phosphoinositide signaling system in the development of pathologic cardiac hypertrophy and heart failure (D'Angelo et al., 1997; Mende et al., 1998; Hunter et al., 1999). Gq protein-coupled receptor (GPCR) agonists such as angiotensin II (Sadoshima et al., 1993; Oro et al., 2007), endothelin-1 (Shubeita et al., 1990), and phenylephrine (Otani et al., 1988) activate phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate, which causes a transient increase in levels of cellular diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (Figure 1). DAG functions as a potent activator of protein kinase C (PKC). The binding of DAG to the C1 domain of PKC induces an active conformation, and activated PKC regulates a variety of cellular functions including cell growth and differentiation (Nishizuka 1988; Nishizuka 1992). It has been previously demonstrated that PKC plays an important role in the development of cardiac hypertrophy and progression to heart failure (Takeishi et al., 1998; Bowling et al., 1999; Takeishi et al., 1999; Takeishi et al. 2000). One major route for terminating DAG signaling is thought to be its phosphorylation and inactivation by DAG kinase (DGK)

producing phosphatidic acid (Goto & Kondo, 2004; Luo et al., 2004; Goto et al., 2006). One of the best known functional roles of DGK is to regulate PKC activity through DAG metabolism (Topham et al., 1998; Topham & Prescott, 1999). However, recent reports have suggested that the functional significance of DAG is not restricted to PKC pathway, and DAG also activates several proteins including Ras GRP (Ebinu et al., 1998), protein kinase D (Baron & Malhotra, 2002), and transient receptor potential proteins (Hofmann et al., 1999). These data suggest that DAG is more widely implicated in cellular events and cellular DAG level is strictly controlled to maintain normal physiological conditions (Figure 1). In addition, phosphatidic acid produced by DGK has signaling functions and serves as a lipid second messenger to regulate a variety of signaling proteins including PKC ζ (Limatola et al., 1994) and phospholipase C γ 1 (Jones & Carpenter, 1993). Therefore, DGK is one of the key enzymes closely involved in lipid-mediated cellular signaling events by attenuation of DAG and production of phosphatidic acid (Topham, 2006; Wattenberg et al., 2006).

2. Expressions of DGK isoforms in the heart

2.1. The mammalian DGK family

DGK α was firstly cloned from porcine thymus cDNA library (Sakane et al., 1990).

This contains two EF bands and two cysteine-rich zinc finger motifs in addition to the

catalytic domain in the primary structure. To date, ten DGK isoforms have been identified in mammals such as DGK α , β , γ , δ , ϵ , ζ , η , θ , ι , and κ (Goto & Kondo, 2004; Luo et al., 2004; Imai et al., 2005; Goto et al., 2006). All of the mammalian DGK isoforms contain a conserved catalytic domain and zinc finger motifs rich in cysteine residues. The zinc finger motifs in DGK resemble C1 domains found in PKC. In the presence of zinc ions, C1 domains of PKC bind DAG and its analogues, phorbol esters, which activate PKC. DGK α , β , and γ belong to the class I family characterized by the presence of a Ca²⁺-binding domain (EF hand). EF hands bind Ca²⁺ with different affinities and induce Ca²⁺-dependent conformational changes (Yamada et al., 1997). DGK δ and η belong to the class II that contains pleckstrin homology domains. The pleckstrin homology domain interacts with phosphatidylinositol 4,5-bisphosphate, inositol phosphate, PKC, and $\beta\gamma$ -subunit of heterotrimeric G proteins (Sakane & Kanoh, 1997). DGK ϵ belongs to the class III, and is highly selective for DAG containing arachidonate as the *sn*-2 fatty acyl moiety (Walsh et al., 1994). Class IV includes DGK ζ and ι , characterized by a nuclear localization signal and ankyrin repeats. The nuclear localization signal sequence overlaps with a motif similar to the phosphorylation site domain of the myristoylated alanine-rich C-kinase substrate proteins (Bunting et al., 1996). The ankyrin repeat motif is involved in protein-protein interactions (Blank et al., 1992). Class V includes DGK θ characterized by the presence of zinc finger

motifs, a protein-rich region at the N-terminus, and a pleckstrin homology domain in the middle of the structure that partially overlaps with a putative Ras-associated domain (Houssa et al., 1997). In mammals, DGK isoforms are detected in various tissues and cell types, suggesting the importance of this kinase in basic cellular functions.

2.2. DGK isoform expression in the heart after myocardial infarction

Although DGK isoforms are abundantly expressed in the brain, Takeda et al. firstly investigated expression of DGK isoforms in rat hearts under a normal condition and after myocardial infarction by in situ hybridization histochemistry and immunohistochemistry (Takeda et al., 2001). In normal left ventricular myocardium, DGK α , DGK ϵ , and DGK ζ mRNAs are expressed, and DGK ζ is the predominant isoform. In infarcted hearts, the expression of DGK ζ is enhanced in the peripheral zone of the necrotic area and at the border zone at 3 and 7 days after myocardial infarction. However, they mentioned the possibility that the enhanced DGK ζ expression in the infarcted and border areas could be attributed to granulocytes and macrophages infiltrated in the myocardium (Takeda et al., 2001). In contrast, the DGK ϵ expression is decreased in the left ventricle after myocardial infarction. These results suggest that each DGK isoform might have different functional characteristics in the healing and left ventricular remodeling after myocardial infarction.

2.3. Changes in DGK isoform expression in the heart by pressure-overload

Yahagi et al. examined expressions of DGK isoforms under chronic pressure-overload induced by aortic banding in rats (Yahagi et al., 2005). The expressions of DGK ϵ mRNA, but not DGK ζ mRNA, in the left ventricular myocardium decreased in the aortic banded rats compared with sham-operated rats. They also investigated changes in subcellular localization of the DGK ζ isoform by Western blotting using isoform specific antibody. DGK ζ protein in the left ventricular myocardium translocated from the particulate to the cytosolic compartment in the aortic banded rats. These results suggest that DGK ϵ and DGK ζ play distinct roles in the development of cardiac hypertrophy by pressure-overload and that the two isoforms are differentially regulated.

2.4. DGK ζ mRNA expression after ET-1 stimulation

DGK ζ is the first nuclear isoform identified and this was confirmed by cDNA transfection into cultured COS cells (Goto and Kondo, 1996). DGK ζ associates with a variety of molecules via the C-terminal ankyrin repeats and PDZ-binding motif. DGK ζ is widely and abundantly expressed throughout the body including the heart, and Takahashi et al. examined changes in DGK ζ mRNA expression after endothelin-1 (ET-1) stimulation in

cultured neonatal rat cardiomyocytes by real-time RT-PCR analysis (Takahashi et al., 2005). DGK ζ mRNA level increased significantly at 1 hour and it peaked at 3 hours after ET-1 stimulation. However, expression of DGK ζ returned to the basal level after 6 hours. ET-1 upregulated DGK ζ mRNA expression in rat neonatal cardiomyocytes. To identify which ET receptor subtype was responsible for the induction of DGK ζ mRNA, they also examined effects of selective antagonists for ET_A (BQ123) and ET_B (BQ788) on ET-1-induced DGK ζ mRNA expression (Takahashi et al., 2005). ET-1-induced increases in DGK ζ mRNA expression were significantly inhibited by treatment with BQ123, but not by BQ788. These observations suggest that ET-1 increases DGK ζ mRNA expression via the ET_A receptor in cardiomyocytes.

3. Regulatory role of DGK ζ in cultured neonatal rat cardiomyocytes

3.1. DGK ζ blocks ET-1-induced PKC ϵ translocation and ERK activation

Although the functional role of DGK has been well characterized in the central nervous system, the functional significance of the DGK family has not been rigorously examined in the cardiovascular system. To examine the effect of DGK ζ on subcellular signaling in the cardiomyocyte, a recombinant adenovirus encoding for rat DGK ζ was used (Takahashi et al., 2005). It has been reported that ET-1, a potent hypertrophic agonist,

causes the translocation of PKC ϵ to the membrane fraction in rat neonatal cardiomyocytes (Clerk et al., 1994). Therefore, Takahashi et al. examined the effects of DGK ζ or LacZ on ET-1-induced translocation of PKC isoforms in cardiomyocytes using isoform-specific antibodies (Takahashi et al., 2005). The membrane-associated immunoreactivity of the PKC ϵ isoform was significantly increased in ET-1-stimulated cardiomyocytes after LacZ transfection. However, ET-1-induced translocation of PKC ϵ was blocked after DGK ζ transfection.

They next investigated effects of DGK ζ on ET-1-induced extracellular signal regulated kinase (ERK) activation. ERK activation was observed in ET-1-stimulated cardiomyocytes after LacZ transfection. However, after DGK ζ transfection, ET-1-induced ERK activation was completely abolished. These results suggest an inhibitory effect of DGK ζ on ET-1-induced PKC ϵ translocation and ERK activation in cardiomyocytes.

3.2. Inhibition of ET-1-induced AP1 DNA-binding activity by DGK ζ

ET-1-induced activation of the PKC ϵ -ERK pathway leads to activation of the activator protein-1 transcription factor thereby promoting the transcription of immediate-early genes such as c-fos and c-jun (Takahashi et al., 2004). Therefore, Takahashi et al. investigated whether DGK ζ can inhibit ET-1-induced activation of activator protein-1

DNA-binding activity by luciferase reporter assay (Takahashi et al., 2005). The activator protein-1 DNA-binding activity was significantly increased in ET-1-stimulated cardiomyocytes. After transfection of DGK ζ , ET-1 failed to increase activator protein-1 DNA-binding activity. These results suggest an inhibitory effect of DGK ζ on ET-1-induced activator protein-1 DNA-binding activity.

3.3. Effects of DGK ζ on hypertrophic responses to ET-1

Takahashi et al. also examined the effects of DGK ζ or LacZ on hypertrophic responses to ET-1 determined by the induction of hypertrophic gene atrial natriuretic factor (ANF), protein synthesis, and increases in cardiomyocyte surface area (Takahashi et al., 2005). Real-time RT-PCR revealed that ET-1 induced ANF gene expression after LacZ transfection. However, DGK ζ inhibited ET-1-induced increases in ANF gene expression levels. The protein synthesis was evaluated by the incorporation of [³H]-leucine into cultured cardiomyocytes. Although ET-1 augmented [³H]-leucine uptake in control cardiomyocytes, ET-1 did not increase [³H]-leucine uptake in cardiomyocytes infected with DGK ζ . Furthermore, they measured cardiomyocyte surface areas after DGK ζ or LacZ transfection. ET-1 stimulation for 48 hours caused enlargement of cardiomyocyte surface area infected with LacZ (Takahashi et al., 2005). However, after transfection of DGK ζ ,

ET-1 did not cause increases in cardiomyocyte surface area. These results suggest that DGK ζ blocks hypertrophic responses by ET-1.

4. Functional role of DGK ζ in *in vivo* mouse hearts

4.1. GPCR agonist-induced cardiac hypertrophy

4.1.1. DGK ζ blocks GPCR agonist-induced activation of the DAG-PKC signaling

To examine the functional role of DGK ζ in *in vivo* hearts, Arimoto et al. generated a a transgenic mouse with cardiac specific overexpression of DGK ζ (DGK ζ -TG) using an α -myosin heavy chain (MHC) promoter (Arimoto et al., 2006). DGK ζ -TG and wild type littermate (WT) mice were assessed with respect to their susceptibility to hypertrophic response to subpressor doses of subcutaneous angiotensin II (Harada et al., 1998) and phenylephrine (Asakura et al., 2002) administration. No significant changes in body weight, heart rate and blood pressure were observed between WT and DGK ζ -TG mice after subcutaneous infusion of angiotensin II or phenylephrine. As Paul et al. previously demonstrated angiotensin II-induced translocation of PKC isoforms through pathways involving phospholipase C in the *ex vivo* guinea pig heart (Paul et al., 1997), the membrane-associated immunoreactivity of PKC α and PKC ϵ , but not PKC β and PKC δ , were significantly increased in angiotensin II-treated WT mice compared with saline-infused WT

mice. However, angiotensin II-induced translocation of PKC α and PKC ϵ were blocked in DGK ζ -TG mice. These results are consistent with *in vitro* data showing that DGK ζ spatially regulates PKC α activity by attenuating local accumulation of DAG in HEK293 cells (Luo et al., 2003). Next, they examined effects of another GPCR agonist, phenylephrine, in DGK ζ -TG and WT mice. Phenylephrine induced translocation of the PKC ϵ isoform, but not α , β , and δ isoforms in WT mouse hearts. However in DGK ζ -TG mice, translocation of the PKC ϵ by phenylephrine was completely blocked. These data suggested that DGK ζ has an inhibitory effect on GPCR agonist-induced translocation of PKC isoforms in *in vivo* mouse hearts.

Then, lipid extracts were prepared from the left ventricular myocardium, and Arimoto et al. quantified myocardial DAG levels in WT and DGK ζ -TG mouse hearts (Arimoto et al., 2006). At basal condition, DAG levels were similar between WT and DGK ζ -TG mice. In WT mouse hearts, myocardial DAG level increased significantly after continuous administration of phenylephrine for 3 days. On the other hand, this effect of phenylephrine on myocardial DAG levels was completely suppressed in DGK ζ -TG mouse hearts. These data support the notion that DGK ζ regulates PKC activity by controlling cellular DAG levels.

4.1.2. Effects of DGK ζ on hypertrophic programs in response to GPCR agonists

Ventricular hypertrophy induced by continuous infusion of angiotensin II or phenylephrine is accompanied by the induction of several specific genes such as ANF (Harada et al., 1998; Takahashi et al., 2005). The mRNA expression of ANF was increased in WT mice given angiotensin II and phenylephrine compared to saline-infused WT mice (Arimoto et al., 2006). However in DGK ζ -TG mice, angiotensin II failed to cause gene induction of ANF. Phenylephrine-induced ANF gene induction was also blocked in DGK ζ -TG mice. These data suggest that DGK ζ blocks hypertrophic gene induction by angiotensin II and phenylephrine in *in vivo* mouse hearts.

Heart weight and left ventricular weight corrected for body weight were not significantly different between saline-infused WT and saline-infused DGK ζ -TG mice. Subcutaneous infusion of angiotensin II and phenylephrine caused significant increases in ratios of: a) heart to body weight; and b) left ventricle to body weight in WT mice. However in DGK ζ -TG mice, neither angiotensin II nor phenylephrine produced increases in ratios of the heart to body weight and ratios of the left ventricle to body weight.

Microscopic observations revealed that no significant difference in cardiomyocyte cross sectional area was seen between saline-infused WT and saline-infused DGK ζ -TG mice.

In WT mice, cardiomyocyte cross sectional area was significantly increased by angiotensin II and phenylephrine infusion. However in DGK ζ -TG mice, neither angiotensin II nor phenylephrine caused increases in cardiomyocyte cross sectional area. Taken together, these data clearly demonstrate that DGK ζ might interfere with GPCR agonist-induced cardiac hypertrophy.

4.2. Pressure overload-induced cardiac hypertrophy

4.2.1. Cardiac hypertrophy after tranverse aortic constriction in DGK ζ -TG mice

In the clinical setting, mechanical stress is more relevant and important as a cause of cardiac hypertrophy. Therefore, to examine the effects of DGK ζ on mechanical stress-induced cardiac hypertrophy, Harada et al. performed transverse aortic constriction (TAC) in DGK ζ -TG and WT mice (Harada et al., 2007). Extensive cardiac hypertrophy due to pressure-overload was observed at 4 weeks after TAC in WT mice. However in DGK ζ -TG mice, cardiac hypertrophy after TAC was significantly attenuated. At 4 weeks after TAC surgery, the ratio of heart weight to body weight was significantly lower in DGK ζ -TG mice than in WT mice.

Microscopic observations revealed that no significant difference in cardiomyocyte cross sectional area was seen between sham-operated WT mice and sham-operated DGK ζ -TG

mice. Cardiomyocyte cross sectional area was increased in WT mice at 4 weeks after TAC. However, increases in cardiomyocyte cross sectional area after TAC were significantly attenuated in DGK ζ -TG mice compared to WT mice.

Echocardiography was performed at baseline and 4 weeks after TAC or sham surgery in WT and DGK ζ -TG mice. Interventricular septal wall thickness and left ventricular end-diastolic dimension were significantly increased in WT mice after TAC. However in DGK ζ -TG mice, the increase in interventricular wall thickness after TAC was attenuated compared to WT mice. Moreover, the reduction of left ventricular fractional shortening at 4 weeks after TAC was attenuated in DGK ζ -TG mice compared to WT mice.

4.2.2. Expressions of fetal genes in hypertrophied hearts

Harada and colleagues (Harada et al, 2007) next examined mRNA expression of fetal type genes such as ANF and β -MHC at 4 weeks after TAC in WT and DGK ζ -TG mice by real-time RT-PCR. Expression of ANF was markedly up-regulated in WT mice after TAC. Conversely in DGK ζ -TG mice, gene induction of ANF in responses to TAC was significantly attenuated compared to WT mice. The β -MHC/ α -MHC ratio was also markedly increased in WT mice after TAC, but this response was blunted in DGK ζ -TG mice.

4.2.3. Myocardial fibrosis and expressions of profibrotic genes

Since reactive interstitial and perivascular fibrosis adversely alters myocardial stiffness and ultimately leads to left ventricular dysfunction (Kuwahara et al., 2004), Harada et al. examined myocardial fibrosis at 4 weeks after TAC in WT and DGK ζ -TG mice using sections stained with Masson trichrome (Harada et al., 2007). Prominent fibrosis was observed in WT mice at 4 weeks after TAC as reported previously (Takimoto et al., 2005). However, the degree of myocardial fibrosis was much less in DGK ζ -TG mice than in WT mice at 4 weeks after TAC. These results indicated that, although pressure-overload caused fibrous formation in the left ventricular myocardium, reactive fibrosis was significantly attenuated by DGK ζ .

Harada et al. next examined expression of profibrotic genes such as transforming growth factor (TGF)- β 1, collagen type I, and collagen type III to investigate whether these morphological observations were accompanied by alterations in gene expression relevant to fibrotic changes (Lijnen et al, 2000; Manabe et al., 2002). Real-time RT-PCR revealed that TGF- β 1, collagen type I, and collagen type III mRNA at 4 weeks after TAC were up-regulated in WT mice. However, these increases in collagen type I and type III mRNA, but not TGF- β 1, were significantly attenuated in DGK ζ -TG mice compared to WT mice.

4.3. Ventricular remodeling after myocardial infarction

4.3.1. Effects of DGK ζ on left ventricular remodeling after myocardial infarction

Niizeki et al. examined effects of DGK ζ on left ventricular remodeling after myocardial infarction in DGK ζ -TG mice (Niizeki et al., 2007). Left anterior descending coronary artery was ligated, and mice were sacrificed 4 weeks later. At 4 weeks after myocardial infarction, the ratio of heart weight to body weight and the ratio of left ventricular weight to body weight were significantly lower in DGK ζ -TG mice than in WT mice. Lung weight and right ventricular weight at 4 weeks after myocardial infarction were also significantly lower in DGK ζ -TG mice than in WT mice, although infarct size at 4 weeks after coronary ligation was similar between WT and DGK ζ -TG mice.

Echocardiography was performed at baseline, 1 week, and 4 weeks after surgery in WT and DGK ζ -TG mice. Left ventricular end-diastolic dimension was significantly larger in WT mice at 1 week after myocardial infarction compared to sham-operated WT mice, and left ventricular end-diastolic dimension was further dilated at 4 weeks after myocardial infarction. However, ventricular dilatation after myocardial infarction was prevented in DGK ζ -TG mice compared to WT mice. Moreover, the reduction of left ventricular fractional shortening after myocardial infarction was attenuated in DGK ζ -TG mice compared to WT mice at 4 weeks after coronary artery ligation.

4.3.2. *Translocation of PKC isoforms, expression of fetal genes and cardiomyocyte hypertrophy*

Niizeki and colleagues examined translocation of PKC isoforms in the non-infarct area of DGK ζ -TG and WT mice (Niizeki et al., 2007). They detected translocation of PKC α , β , δ , and ϵ isoforms at 4 weeks after myocardial infarction in the non-infarct area of WT mouse hearts. However in DGK ζ -TG mice, translocation of PKC α and PKC ϵ , but not β and δ , were significantly attenuated.

Niizeki et al. next examined mRNA expressions of fetal type genes such as ANF and β -MHC in the non-infarct area at 4 weeks after myocardial infarction in WT and DGK ζ -TG mice. Expressions of ANF and β -MHC were up-regulated in WT mice after myocardial infarction compared to WT sham mice. Conversely in DGK ζ -TG mouse hearts, gene induction of ANF and β -MHC in responses to myocardial infarction was significantly attenuated compared to WT mice.

Microscopic observations revealed that no significant difference in cardiomyocyte cross sectional area was seen between sham-operated WT and DGK ζ -TG mice. Cardiomyocyte cross sectional area in the non-infarct area was profoundly increased in WT mice at 4 weeks after myocardial infarction. However, increases in cardiomyocyte cross

sectional area after myocardial infarction was significantly attenuated in DGK ζ -TG mice compared to WT mice.

4.3.3. DGK ζ inhibits myocardial fibrosis in the non-infarct area

Since reactive interstitial and perivascular fibrosis in the non-infarcted area adversely alters myocardial stiffness and ultimately leads to left ventricular dysfunction (Lijnen et al., 2000; Shishido et al., 2003), Niizeki et al. examined myocardial fibrosis in the non-infarct area at 4 weeks after myocardial infarction in WT and DGK ζ -TG mice using sections stained with van Gieson stain (Niizeki et al., 2007). In the non-infarct area, prominent perivascular and interstitial fibrosis was observed in WT mice at 4 weeks after myocardial infarction as reported in previous studies (Shishido et al., 2003). However, the degree of myocardial fibrosis at the non-infarct area was much less in DGK ζ -TG mice than in WT mice at 4 weeks after myocardial infarction. These results indicated that, although coronary ligation caused fibrous formation in the infarct area and the size of myocardial infarction was similar between WT and DGK ζ -TG mouse hearts, reactive fibrosis in the non-infarct area was significantly attenuated by DGK ζ .

Niizeki et al. next examined expressions of profibrotic genes such as TGF- β 1, collagen type I, and collagen type III to investigate whether these morphological observations

were accompanied by alterations in gene expressions relevant to fibrotic changes. Real-time RT-PCR revealed that TGF- β 1, collagen type I, and collagen type III mRNA in the non-infarct area at 4 weeks after myocardial infarction were markedly up-regulated in WT mice. However, these responses were significantly attenuated in DGK ζ -TG mice compared to WT mice.

4.3.4. Survival rates after myocardial infarction in DGK ζ -TG mice

Myocardial infarction was created by coronary artery ligation in 93 WT mice and 82 DGK ζ -TG mice (Niizeki et al., 2007). The rate of acute surgical death was not different between WT mice and DGK ζ -TG mice. The survival rate until 4 weeks after myocardial infarction was significantly higher in DGK ζ -TG mice (66%) than in WT mice (45%). The major cause of death in the first 10 days after myocardial infarction was cardiac rupture, and the incidence of cardiac rupture was not different between WT and DGK ζ -TG mice. Thus, these differences in survival were due to less lethal congestive heart failure in DGK ζ -TG mice than in WT mice.

5. Regulation of T cell activity by DGK ζ

It has been widely recognized that T cell receptor engagement leads to T cell

activation (Kisielow et al. 1995; Kane et al, 2000). T cell development in the thymus and activation of mature T cells in the periphery depend on signals stimulated by engagement of the T cell receptor. Diacylglycerol generated in response to T cell receptor stimulation activates Ras-GRP and protein kinase C θ in T cells. Zhong et al. showed in Jurkat T cells that DGK ζ interferes with T cell receptor-induced Ras and ERK activation, activator protein-1 induction, and expression of the activation marker CD69 (Zhong et al., 2002).

Zhong et al. generated DGK ζ -deficient mice and showed that DGK ζ -deficient T cells are hyperresponsive to T cell receptor stimulation *in vivo* (Zhong et al., 2003). Furthermore, DGK ζ -deficient mice mounted a more robust immune response to lymphocytic choriomeningitis virus infection than did WT mice. These results demonstrate the importance of DGK ζ as a physiological negative regulator of T cell receptor signaling and T cell activation.

6. Conclusion

Three DGK isoforms, α , ϵ , and ζ are expressed in cardiac myocytes, and DGK ζ is the predominant isoform. DGK ζ acts as a regulator of GPCR signaling by controlling cellular DAG levels and PKC activity. DGK ζ blocks cardiac hypertrophy by GPCR agonists and pressure-overload, attenuates ventricular remodeling and improves survival after

myocardial infarction *in vivo*. These data provide a novel insight for subcellular mechanisms of heart failure, and DGK ζ may be a new therapeutic target to prevent cardiac hypertrophy and progression to heart failure. The functional roles of two other isoforms, α and ε , in the heart remain to be elucidated.

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Figure legend

Figure 1. Functional roles of DAG and DGK in the $G\alpha_q$ -coupled receptor signaling.

