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Review

Role of Diacylglycerol Kinases in Glucose and Energy Homeostasis

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Diacylglycerol kinases (DGKs) catalyze a reaction that converts diacylglycerol (DAG) to phosphatidic acid (PA). DAG and PA act as intermediates of *de novo* lipid synthesis, cellular membrane constituents, and signaling molecules. DGK isoforms regulate a variety of intracellular processes by terminating DAG signaling and activating PA-mediated pathways. The ten DGK isoforms are unique, not only structurally, but also in tissue-specific expression profiles, subcellular localization, regulatory mechanisms, and DAG preferences, suggesting isoform-specific functions. DAG accumulation has been associated with insulin resistance; however, this concept is challenged by opposing roles of DGK isoforms in the development of type 2 diabetes and obesity despite elevated DAG levels. This review focuses on the tissue- and isoform-specific role of DGK in glucose and energy homeostasis.

Highlights

DGKs regulate several cellular processes by controlling the levels of DAG and PA.

Dysregulation of certain DGK isoforms leads to DAG-activation of PKC, attenuation of insulin signaling, and dysregulation of glucose metabolism.

Decreased DGKδ abundance in skeletal muscle of type 2 diabetic patients may contribute to the development of peripheral insulin resistance.

Whole-body deletion of DGKε favors lipid utilization to preserve insulin sensitivity in rodents.

Knockout of DGKζ prevents the development of diet-induced obesity in rodents.

DGK isoforms differentially influence energy homeostasis in a tissue-specific manner.

Generation and Roles of Diacylglycerol and Phosphatidic Acid

Fatty acids are essential for cell structure and metabolism. Several fatty acid classes exist and each lipid class consists of numerous species characterized by chain length and degree of saturation [1]. Fatty acids are involved in three main specialized cellular functions, namely membrane composition, signal transduction, and energy storage. Therefore, alterations in lipid metabolism or distribution of specific lipid species can have profound effects on cellular processes and potentially promote the pathogenesis of metabolic disease [2].

Diacylglycerols (DAGs) are neutral lipids acting as intermediates in fatty acid metabolism, cellular membrane constituents, and key signaling molecules. Generation of DAG occurs through several pathways at different subcellular compartments [3]. Different classes of enzymes are responsible for the intracellular production of DAG (Figure 1). Hydrolysis of phosphatidylinositol or phosphatidylcholine by phospholipase C (PLC) produces DAG. Esterification of monoacylglycerol by acyltransferases and hydrolysis of triacylglycerol by lipases also generates DAG. Moreover, DAG is synthesized from phosphatidic acid (PA) after removal of phosphate by PA phosphatases. Conversely, DAG is metabolized through different enzymatic systems, including re-esterification to triacylglycerol by DAG-acyltransferases (DGATs), hydrolysis to monoacylglycerol by lipases (e.g., hormone-sensitive lipase; HSL), and conversion to phospholipids by phosphotransferases (e.g., diacylglycerol cholinephosphotransferase; CPT) [3]. Additionally, degradation of DAG occurs through phosphorylation by diacylglycerol kinases (DGKs), generating PA in an ATP-dependent reaction.

DAG and PA function as key second messengers that carry out specific tasks for a wide variety of biological processes. DAG regulates cellular functions primarily through binding to C1 protein domains (cysteine-rich zinc finger structures) [4]. The main signaling function of DAG involves the recruitment and activation of classical and novel protein kinase (PK)C isoforms [5,6] (Figure 1). DAG also acts through other downstream effectors by binding C1 domains such as RasGRPs (guanine-nucleotide-releasing proteins) [7] and PKD [8]. Conversely, PA binds to key cellular

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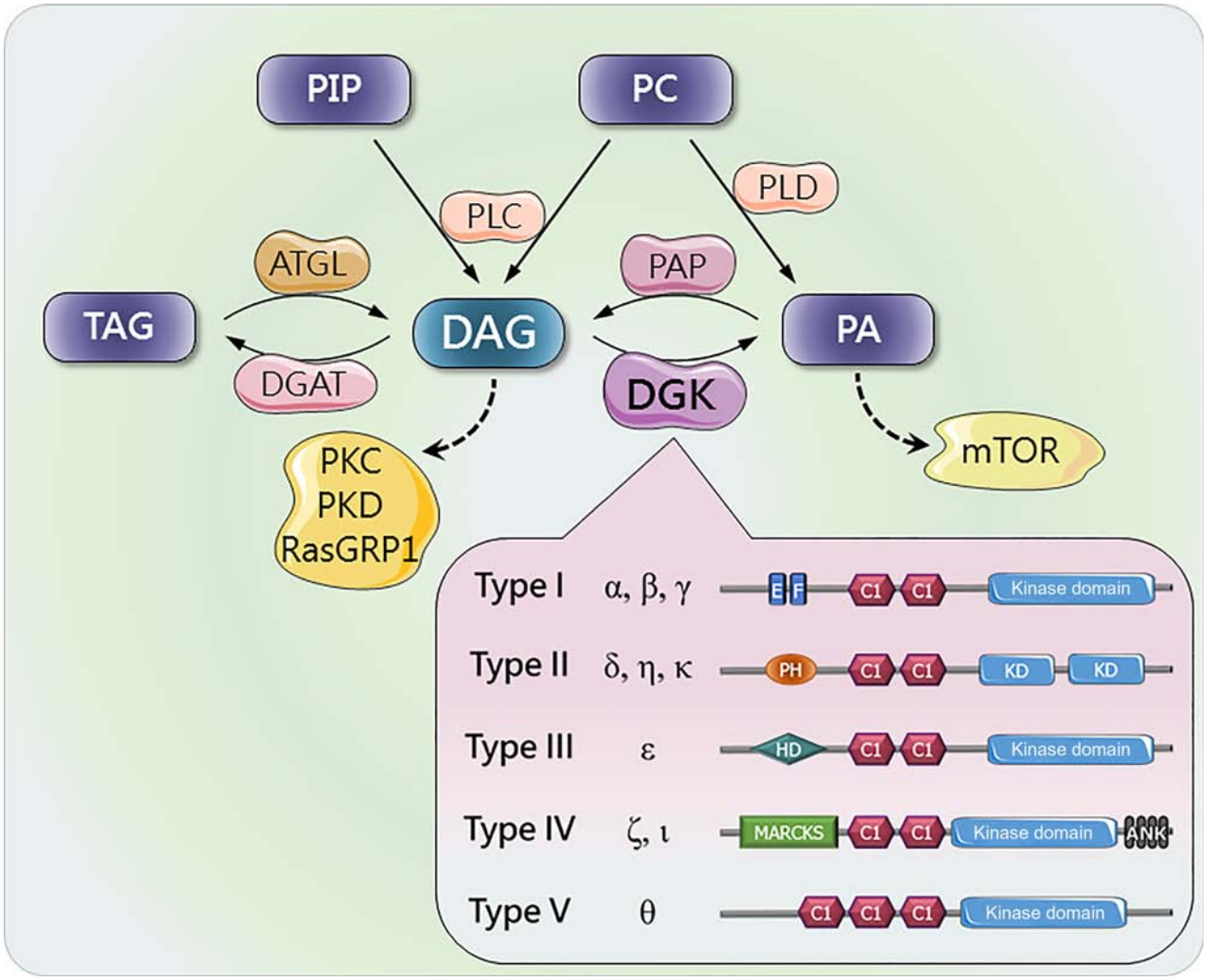

Figure 1. A Central Role for Diacylglycerols (DAGs) as Lipid Intermediates and Second Messengers and Diacylglycerol Kinase (DGK) Enzymes in Fatty Acid Metabolism. DAGs are synthesized intracellularly from specific lipid precursors by different enzymatic systems. DAGs are liberated from inositol phospholipids (PIPs) and other phospholipids including phosphatidylcholine (PC) by the action of phospholipase C (PLC) and phospholipase D (PLD). Phosphatidic acid (PA) hydrolysis by phosphatidic acid phosphatase (PAP) also generates DAG species. Triacylglycerol (TAG) hydrolysis by triglyceride lipases, such as adipose triglyceride lipase (ATGL), and esterification of monoacylglycerides through the monoacylglycerol acyltransferase pathway produces DAG. Conversely, DAGs are intermediates of *de novo* lipid synthesis catalyzed by diacylglyceride acyltransferase (DGAT), and are phosphorylated into PA by DGK. DAG and PA act as second messengers and regulate a variety of cellular functions by binding to specific proteins. Thus, DGK enzymes play a central role in the regulation of lipid signaling by either terminating DAG or producing PA. Ten mammalian DGK enzymes are classified into five subtypes based on the similarity of their structural motifs. Abbreviations: ANK: Ankyrin repeat; C1, cysteine-rich domain; EF, EF-hand motif; HD, hydrophobic domain; MARCKS, myristoylated alanine rich protein kinase C substrate phosphorylation site; mTOR, mammalian target of rapamycin; PH, pleckstrin homology domain.

effectors, such as **mammalian target of rapamycin** (**mTOR**, see Glossary), thereby regulating the activity of the enzyme (Figure 1). mTOR is typically found in mTOR complex (mTORC)1 in association with Raptor, and mTORC2 in association with Rictor. Both mTORC1 and mTORC2 are activated by PA [9]. PA has a stronger affinity for mTORC2 than mTORC1, suggesting that mTORC1 may readily dissociate from PA and therefore be more sensitive to cellular PA levels [9]. Therefore, DGK controls numerous signaling pathways by simultaneously terminating DAG-induced signaling while activating PA-mediated pathways.

DAG accumulation has been linked to the development of lipid-induced **insulin resistance**. In healthy people, insulin activates PKC, which sequentially phosphorylates insulin receptor substrate (IRS), leading to the dissociation of IRS from the insulin receptor, thereby terminating insulin signaling. Therefore, DAG-induced activation of PKC isoforms leads to a negative feedback control mechanism on insulin signaling and attenuates glucose uptake [10]. In skeletal muscle from people with **type 2 diabetes**, DAG content is increased and DGK activity is decreased, leading to impaired PKC activation, insulin signaling, and glucose metabolism [11]. Skeletal muscle DAG accumulation activates PKCθ, PKCβII, and PKCδ, and concomitantly inhibits insulin-mediated IRS1 phosphorylation [12,13]. Hepatic DAG accumulation specifically induces the recruitment of PKCε to the plasma membrane, and concomitantly inhibits insulin-mediated IRS2 phosphorylation [14]. Thus, several lines of evidence suggest that DAG-induced PKC isoform activation impairs insulin signaling and thereby contributes to insulin resistance associated with metabolic disease. Even though the DAG–PKC axis has been extensively studied in the pathogenesis of type 2 diabetes [15], not all DAG isomers activate PKC [16]. Thus investigation of the enzymatic systems regulating the intracellular DAG and PA levels, as well as the role and generation of specific DAG species in signal transduction is of relevance.

Overview of the DGK Family

Structure of DGK Isoforms

DGK is expressed in most organisms with higher diversity in mammals, with one DGK enzyme expressed in *Drosophila melanogaster* and up to ten isoforms identified in humans. Based on structural protein motifs, mammalian isoforms can be classified into five subtypes (Figure 1). All isoforms are characterized by a kinase domain (consisting of a catalytic and an accessory domain) and two C1-like domains. The catalytic domain is highly conserved with distinct characteristics within subtypes [17]. Moreover, the catalytic domain requires other motifs for maximal activity, illustrating functional differences among the isoforms [18]. Type I isoforms (DGKα, β, and γ) contain a repeat of two calcium-binding EF motifs. Type II DGK (δ, η, and κ) are characterized by a Pleckstrin homology domain. DGKε, the only type III isoform, has a hydrophobic domain allowing its binding to membranes. Type IV DGK (ζ and ι) share the same myristoylated alanine-rich C kinase substrate (MARCKS) and ankyrin repeat (ANK) motifs mediating cellular localization and protein interaction. Finally, DGKθ, the only member of the type V family, contains three C1 domains. The biological significance of each domain remains unclear, but functional studies have highlighted the potential role and regulation at specific motifs [19]. Alternative splicing of some DGK isoforms has also been described in humans, thereby further increasing their diversity (Box 1) [20,21]. Therefore, a better

Box 1. Splice Variants of DGK

Alternative splicing of certain DGK isoforms has been described in humans, thereby increasing the diversity of this system further [20,21]. Splice variants of DGK isoforms may be tissue-specific. A truncated DGKγ variant lacking 25 amino acids within the catalytic domain is expressed in almost all tissues, while the full-length active DGKγ variant is expressed in retina and brain [97]. A skeletal and cardiac muscle-specific DGKζ splice variant has been characterized by a longer N terminus than the ubiquitously expressed variant [20]. Additional structural domains have also been described, such as DGKη1 and 2 differing by the presence of a protein interaction sterile α-motif (SAM) domain at the C terminus for the η2 variant [89]. While both variants translocate from the cytoplasm to endosomes upon stress stimuli, only DGKη2 can form a heterodimer with DGKδ1 and DGKδ2 [89]. Splice variants of DGK could differ in their intracellular localization. Isoforms of DGKβ exhibit different C-terminal regions leading to one isoform predominantly associated with the plasma membrane, while the other isoform is predominantly cytoplasmic [98]. Alternative splicing also leads to specific subcellular localizations of DGK isoforms in response to diverse stimuli. For example, depending on the N-terminal sequence, DGKδ can either translocate from the cytoplasm to the plasma membrane or remain cytoplasmic upon stimulation [21]. Thus, DGK splice variants may also elicit substrate specificity that is associated with specific signaling events. Consequently, the heterogeneity of the DGK family suggests that its function goes beyond the sole regulation of DAG content. Alternative splicing of DGK isoforms may reflect the importance of DGK isoforms in cellular physiology, as well as the need for a specialized function within a tissue or in response to specific stimuli. While multiple DGK isoforms have been identified that differ in their structural domains or substrates specificity, the precise cellular functions of these isoforms remain to be determined.

Glossary

Akt or protein kinase B: serine/threonine-protein kinase family involved in various cellular processes including glucose metabolism, cell survival, growth, and angiogenesis. The Akt family consists of three isoforms (Akt1, Akt2, and Akt3). Akt1 is primarily involved in cell proliferation and protein synthesis, and Akt2 regulates glucose uptake and metabolism by mediating insulin-induced translocation of the GLUT4 glucose transporter to the cell surface.
AMP-activated protein kinase (AMPK): cellular energy sensor conserved in all eukaryotic cells. AMPK is activated by an increased cellular AMP/ATP ratio. AMPK regulates the activity of a number of key metabolic enzymes through phosphorylation, and promotes energy-producing pathways and inhibits energy-consuming processes.
Endoplasmic reticulum (ER): organelle involved in the production, processing, and transport of proteins and lipids.
Gluconeogenesis: metabolic process leading to the production of glucose from noncarbohydrate precursors.
Glucose Transporter 4 (GLUT4): facilitated glucose transporter expressed in skeletal and cardiac muscles, as well as brown and white adipose tissues. Under basal conditions, GLUT4 resides intracellularly in vesicles, which translocate to the plasma membrane upon insulin stimulation. In skeletal muscle, contraction/exercise/cellular stress also induces GLUT4 translocation.
Insulin Receptor Substrates (IRSs): family of cytoplasmic signaling intermediates involved in insulin-regulated glucose homeostasis. Phosphorylation of IRS by the insulin receptor allows the recruitment of downstream effectors that promote glucose uptake and utilization for energy production.
Insulin resistance: condition in which skeletal muscle, adipose tissue, and liver are insensitive to insulin and therefore glucose homeostasis is disturbed.
Lipogenesis: synthesis of fatty acids from acetyl-CoA molecules.
Lipolysis: hydrolysis of triglycerides into glycerol and nonesterified fatty acid.
Mammalian target of rapamycin (mTOR): serine/threonine-protein kinase that associates with different

understanding of the regulation and activity of the individual DGK isoforms is necessary given the array of substrates and protein interactions that can lead to various signaling events and ultimately to metabolic alterations.

Tissue Distribution of DGK Isoforms

The first characterization of DGK activity was performed on rat brain microsomes [22], with subsequent studies reporting that all DGK isoforms are expressed in specific regions of the brain [23]. Several lines of evidence highlight a role for DGK in brain function and neuronal disorders [24]. Genome-wide association studies have identified several DGK isoforms that are associated with neurological disorders [25]. DGKη expression is increased in patients with bipolar disorder [26], with DGKη knockout mice presenting a hyperactive and anxious behavioral phenotype [27]. While DGK is expressed in all tissues in an isoform-specific pattern [28], the precise role of each isoform within tissues remains largely unknown. Thus, careful mapping of the tissue and subcellular distribution of DGK isoforms may reveal insight into the relative function of each enzyme.

Subcellular Localization of DGK Isoforms

Given that DAG is generated through various processes, the finding that DGK is detected in different subcellular compartments is unsurprising. DGK activity is dependent on extracellular stimuli, binding partners, and DAG availability (Box 2). DGK activity is low in quiescent cells and increases upon stimuli, with different pools of DGK activity existing within the cell. Almost all DGK enzymes translocate from the cytoplasm to the plasma membrane upon stimulation. Inactive DGKα is present in the cytoplasm and translocates to the membrane in an active form following receptor activation by a calcium-dependent mechanism [29]. Yet, DGK isoforms are present within all subcellular structures. Using *in vitro* overexpression approaches, DGKγ was mainly found within the Golgi, whereas DGKε was found to colocalize with the **endoplasmic reticulum (ER)**, and DGKζ was almost exclusively localized within the nucleus [29]. Nuclear localization of DGKζ has been confirmed in rat cervical neurons [30] and C2C12 myoblasts [31], but similar studies are warranted for each DGK isoform to confirm these *in vitro* findings. Structural domains play a role for organelle targeting, such as a small hydrophobic segment present within the

partners to form complexes (mTORC1 and 2) to regulate key metabolic processes. mTOR is activated by extracellular or intracellular stimuli that reflect cellular energy status to regulate glucose and lipid metabolism.
Steatosis: excessive accumulation of lipid due to increased fatty acid synthesis or a defect in fatty acid utilization.
Type 2 diabetes: chronic disease characterized by hyperglycemia due to insulin resistance and altered insulin secretion.

Box 2. DGK Substrate Preference and Activity Regulation

Distinct isomers of DAG are generated depending on the precursor and the enzyme involved [3]. For instance, hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) by PLC generates 1,2-DAG, while hydrolysis of triacylglycerol in adipose tissue by the triglyceride lipase ATGL produces 1,3- and 2,3-DAG [99]. The different isomers of DAG are of direct importance for signal transduction, but also the selectivity of enzymes that generate or degrade DAG. The phosphatidylinositol turnover pathway generates arachidonic acid (20:4)-containing DAG, which is phosphorylated by DGK isoforms, and was previously considered a main source of DAG metabolized by DGK. Until recently, only DGKε exhibited a preference for polyunsaturated DAG *in vitro* [100,101], whereas the other DGK isoforms phosphorylated DAG species independently of their acyl chain. In several cell types, the substrate preference for some DGK isoforms has been subsequently identified. For example, in C2C12 myoblasts, DAG-containing palmitic acid, but not arachidonic acid is preferably metabolized by DGKδ in response to high glucose [102]. Conversely, during neuroblastoma cell differentiation, DGKδ has no impact on the production of PA-containing a dipalmitoyl chain, while DGKζ generates this PA species [103]. These findings suggest that substrate preference of DGKδ is tissue-specific, most likely due to the presence of specific interacting partners, as well as the subcellular localization of the enzyme. Thus, intrinsic substrate preferences remain to be determined for most isoforms and should be carefully examined in a tissue-specific manner. Collectively, tissue-specificity of DGK enzymes indicates that the regulation of the different isoforms and the processes they control are highly complex. Under physiological conditions, DAG is produced in specific cellular compartments and coordinated relocalization of DGK is necessary for DAG degradation, which thereby terminates its signal. Structural domains within the DGK subtypes govern this compartmentalization. Translocation from one compartment to another is thought to be under the control of post-translational modifications or protein–protein interaction. Subcellular localization and activity of DGKα and DGKζ are controlled by phosphorylation, necessary for exclusion from the nucleus [56,104], whereas activity of DGKθ is inhibited by direct interaction with RhoA [105]. Yet, isoform-specific mechanisms that regulate the subcellular enzyme distribution and activity are largely unknown.

N-terminal sequence of DGKε that is necessary for its ER localization [32]. However, most studies characterizing the intracellular localization of DGK isoforms have used fusion recombinant proteins. Therefore, further studies are required to characterize the subcellular localization and function of the different DGK isoforms within native tissues.

Several DGK isoforms are present within the nucleus, though their role within this compartment is poorly characterized. DGKζ is localized within the nucleus, and phosphorylation on its MARCKS domain by PKCα reduces its activity and excludes it from the nucleus, resulting in cell growth inhibition [33]. Nuclear lipids are potent ligands of nuclear receptors that regulate gene expression. However, a role for DAG as a direct ligand within the nucleus remains unknown. Interestingly, loss of DGAT1, an enzyme responsible for the conversion of DAG and fatty acyl-CoA to triacylglycerol, reduces the expression of peroxisome proliferator-activated receptors (PPARα, δ, and γ), and respective target genes, in skeletal and cardiac muscle [34]. This suggests a role for nuclear DAG in PPAR activation and downstream targets involved in lipid metabolism.

The role of DGK isoforms in regulating signaling events by local metabolism of DAG and thus, PA generation within different subcellular localizations remains largely unknown. Dysregulation of DGK isoform expression could potentially alter the abundance of DAG/PA species in specific subcellular organelles. In skeletal muscle, membrane saturated DAG is associated with insulin resistance and PKC activation [35], while in liver, DAG accumulation within cytosolic lipid droplets is associated with PKCε activation and insulin resistance [36]. Whether hepatic DAG-containing lipid droplets constitute an active signaling pool or accumulation of DAG and PKC activation are secondary to the establishment of insulin resistance remains to be determined. Additionally, intramyocellular DAG content is more abundant in sarcolemmal and mitochondrial/ER membrane fractions, accounting for about 85% of total DAG abundance [37]. Consequently, characterization of isoform subcellular localization in a tissue might uncover new roles for DGK isoforms.

DAG Accumulation, DGK, and Whole-Body Insulin Sensitivity

The accumulation of DAG in liver and peripheral organs has been coupled to the development of insulin resistance via DAG activation of PKC and attenuation of insulin signaling and glucose uptake. Intramyocellular levels of DAG are increased in people with type 2 diabetes, and inversely correlated with insulin sensitivity [38,39]. Skeletal muscle DAG accumulation is associated with PKCθ activation in humans, thereby decreasing insulin signaling through IRS1 tyrosine phosphorylation [39]. DGKδ is highly expressed in insulin sensitive tissues such as skeletal muscle and adipose tissue [28,40], and DGKδ gene expression and protein abundance are decreased in skeletal muscle from type 2 diabetic patients [11]. Haploinsufficiency of DGKδ in mice is associated with increased DAG content in skeletal muscle, concomitant with peripheral insulin resistance and metabolic inflexibility [11]. Thus, downregulation of DGKδ may be a key contributor to the accumulation of intramuscular DAG and subsequent development of peripheral insulin resistance (Figure 2).

Conversely, intramuscular DAG content has been shown to be increased in insulin sensitive athletes compared with lean and obese untrained humans [41], while subcellular analysis of DAG localization reveals that accumulation of unsaturated DAG within the ER and mitochondria is inversely correlated with insulin sensitivity [37]. Whole-body deletion of DGKε increases unsaturated and saturated DAG species content in skeletal muscle of diet-induced obese mice, concomitant with improved glucose tolerance [42]. Furthermore, DGKε colocalizes with the ER [32] and decreased activity could favor lipid utilization to preserve insulin sensitivity. Collectively, these studies highlight the importance of localization and saturation of DAG as a key factor for insulin sensitivity, rather than the total amount of DAG within an organ/tissue. Therefore, depending

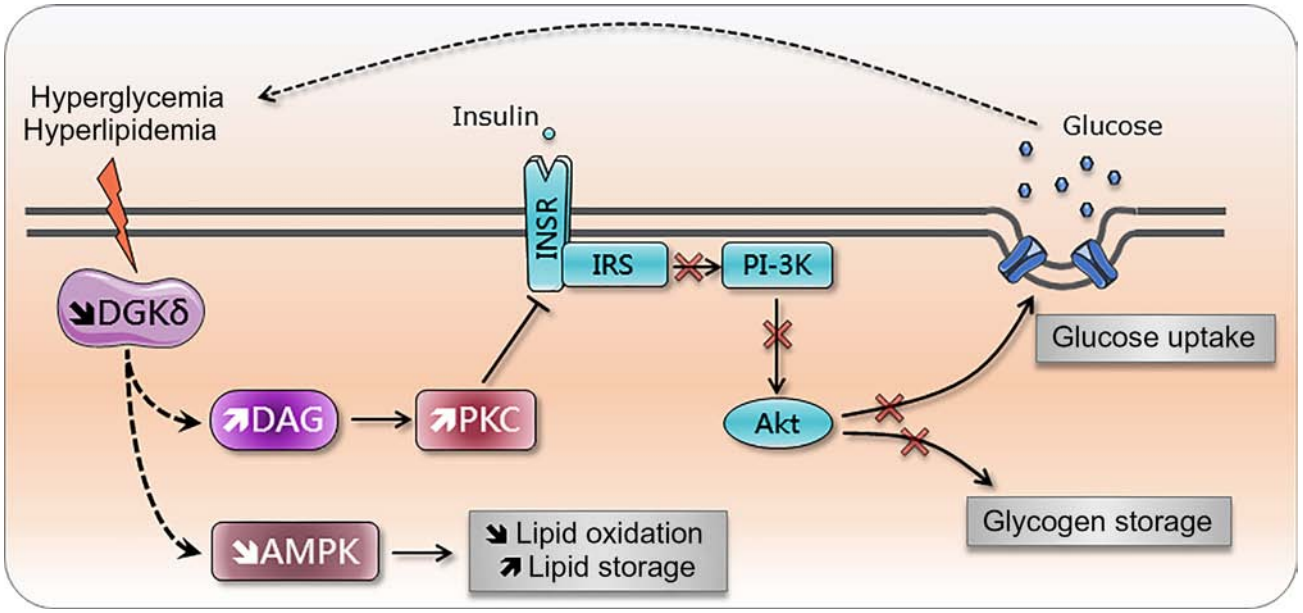

Figure 2. Contribution of Diacylglycerol Kinase (DGK)δ to Pathogenesis of Insulin Resistance. Decreased DGKδ abundance leads to the accumulation of diacylglycerol (DAG). DAG activates protein kinase C (PKC), which inhibits insulin signaling and reduces glucose uptake and glycogen storage in skeletal muscle. Insulin resistance further aggravates hyperglycemia and may trigger a negative feedback loop on DGKδ expression and activity. Decreased DGKδ abundance reduces AMP-activated protein kinase (AMPK) activity, thus favoring lipid storage over oxidation. Abbreviations: INSR, insulin receptor; IRS, insulin receptor substrate; PI-3K, phosphoinositide 3-kinase.

on substrate specificity and subcellular localization, DGK enzymes may exhibit opposite effects on tissue-specific and whole-body insulin sensitivity.

DGKδ and Insulin Resistance

Obesity and insulin resistance are characterized by disturbances in adipose tissue metabolism including decreased lipid uptake and fatty acid storage, increased **lipolysis**, and decreased adipocyte differentiation and expandability [43]. The expression of DGKδ is increased upon adipocyte differentiation and is required for complete adipocyte maturation [44]. DGKδ is highly expressed in adipose tissue [28,40] and its expression is upregulated in adipose tissue of leptin-deficient *ob/ob* mice [28,44]; a mouse model characterized by higher adipogenesis and adipose tissue hyperplasia. Therefore, a reduction of DGKδ expression may affect the capacity of adipose tissue to expand in response to increased nutrient intake, and consequently accelerate ectopic fat storage, leading to the development of insulin resistance and subsequent hyperglycemia and type 2 diabetes. This notion is supported by the development of obesity and insulin resistance later in life in DGKδ haploinsufficient mice [11].

DGKδ haploinsufficiency has been associated with decreased **AMP-activated protein kinase (AMPK)** activity in skeletal muscle and a preference for fatty acid storage over oxidation [45]. In skeletal muscle, AMPK is a key energy sensor regulating substrate utilization. Moreover, reduced AMPK activity is associated with the development of insulin resistance in liver and skeletal muscle [46]. Activity of AMPK is reduced in skeletal muscle exposed to high glucose [47], as well as in liver and skeletal muscle of insulin resistant *ob/ob* mice and *fa/fa* rats [48]. Furthermore, PKC suppresses AMPK activity by inhibitory $Ser^{485/491}$ phosphorylation on AMPK [49]. PKC activity is increased in skeletal muscle of DGKδ haploinsufficient mice, and this may contribute to decreased

AMPK activity in this model [11]. Whether hyperglycemia or PKC activation is responsible for reduced AMPK activation in DGKδ haploinsufficient mice is unclear. Further investigations are warranted to determine the relationship between altered AMPK activity and decreased DGKδ activity in skeletal muscle or adipose tissue and the contribution of this network to the pathogenesis of insulin resistance in type 2 diabetes.

Mechanisms controlling DGKδ expression are unknown. Exposure to myristic acid increases DGKδ expression in C2C12 cells, while conversely, DGKδ protein abundance is decreased by ω-9 fatty acids [50]. Myristic acid-induced DGKδ expression enhances glucose uptake in C2C12 myotubes [51]. Furthermore, acute exposure to high glucose in L6 muscle cells rapidly and transiently increases DGK activity, and this is coupled to reduced PKCα activity and increased **glucose transporter (GLUT)4** translocation to the plasma membrane [52]. Evidence of glucose-induced translocation of DGKδ from the cytoplasm to the plasma membrane in L6 and C2C12 muscle cells [52,53] suggests a role for DGKδ in the regulation of glucose uptake in skeletal muscle. Conversely, DGKδ abundance is reduced in skeletal muscle from type 2 diabetic Goto-Kakizaki rats, and levels are restored upon correction of hyperglycemia [11], suggesting that hyperglycemia may directly downregulate DGKδ expression. Increased DAG content in skeletal muscle of DGKδ haploinsufficient mice is associated with increased phosphorylation of PKCδ and decreased insulin-mediated phosphorylation of IRS1 and **Akt**, and glucose uptake [11]. In HeLa cells, knockdown of PKCα rescues phosphorylation of Akt induced by DGKδ deficiency [54], which indicates tissue-specific PKC signaling is involved. Collectively, these observations suggest that DGKδ expression and activity are closely associated with glucose metabolism and insulin resistance.

Altered DGK activity is associated with reduced Akt phosphorylation. For example, DGKδ silencing in hepatocytes decreases Akt phosphorylation, mainly through DGKδ2. Moreover, DGKδ deficiency also reduces Akt phosphorylation in brain, liver, lung, and skeletal muscle *in vivo* [54]. DGK may affect Akt signaling through decreased phosphorylation at the level of the PA–mTOR or PKC axis. Conversely, PP2A and the PHLPP phosphatases are responsible for Akt dephosphorylation [55]. In DGKδ-deficient cells, PHLPP2 has been shown to dephosphorylate Akt, with DAG generated through PLC acting as a dephosphorylation signal [54]. Thus, DGK isoforms may regulate Akt phosphorylation by affecting either kinase or phosphatase activity. As Akt is distributed within different subcellular compartments [55], the effect of DGK on Akt activity may be subcellular-dependent and controlled by different stimuli.

DGKζ and Regulation of Metabolism

DGKζ has been implicated in the regulation of glucose uptake (Figure 3). DGKζ interacts with enzymes activated by either DAG (Ras-GRP, cPKC) or PA (PIP5K1α), placing DGKζ central within signaling pathways activated by these lipid species [56,57]. In adipocytes, DGKζ regulates GLUT4 translocation to the plasma membrane by interacting directly with IRS1 and PIP5K1α. Under basal conditions, DGKζ associates with IRS1, and inhibits GLUT4 translocation, whereas under insulin-stimulated conditions, IRS1 is released from this complex, and activates the canonical insulin signaling pathway and subsequently increases glucose uptake. Synergistically, the association between DGKζ–PIP5K1α enhances GLUT4 translocation [58]. Insulin-stimulated glucose uptake is enhanced in glycolytic extensor digitorum longus skeletal muscle from high-fat-fed DGKζ knockout mice [59]. Expression profiles of DGK isoforms within insulin sensitive tissues reveal that DGKζ is the predominant isoform in liver and glycolytic skeletal muscle [28], and deletion of DGKζ may protect against the development of insulin resistance in these tissues. Skeletal muscle insulin sensitivity is enhanced in DGKζ knockout mice concomitant with increased intramyocellular DAG content [59]. This further supports the notion that the specific DAG species

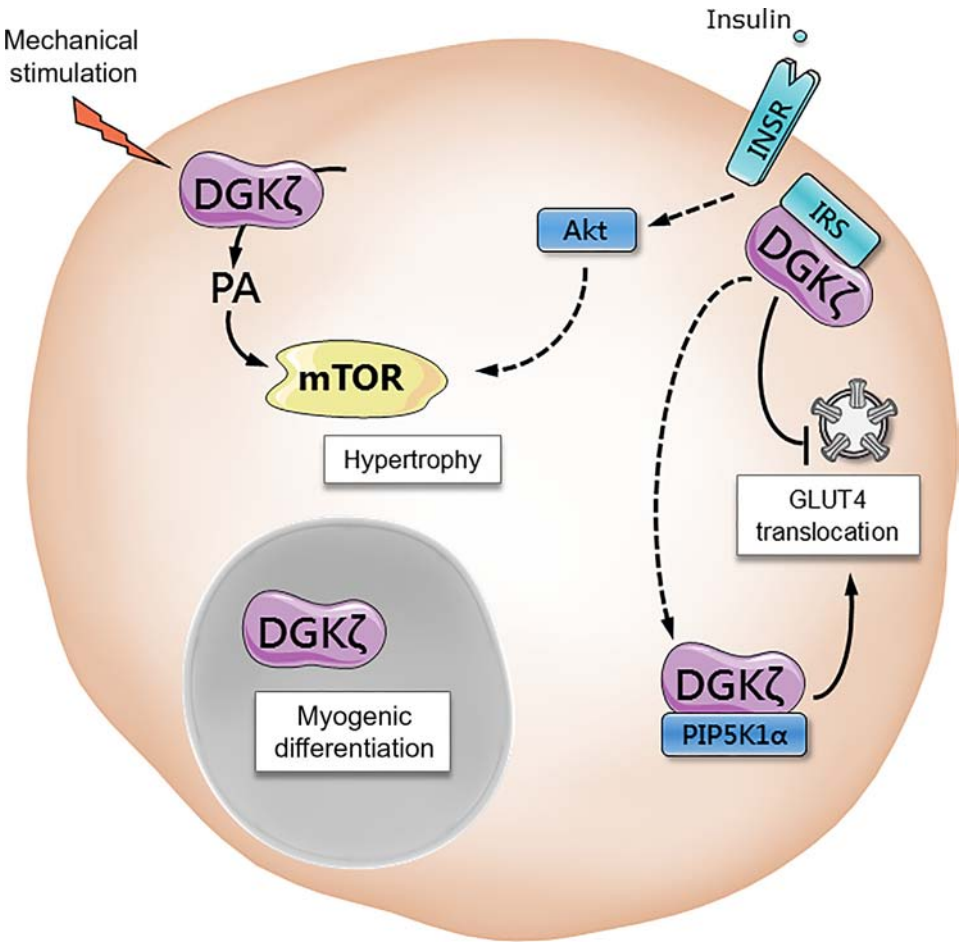

Figure 3. Subcellular Localization of Diacylglycerol Kinase (DGK)ζ and Signaling Pathways. Cytoplasmic production of phosphatidic acid (PA) by DGKζ directly activates mammalian target of rapamycin (mTOR) and enhances protein synthesis and skeletal muscle hypertrophy. Under basal conditions, DGKζ associates with insulin receptor substrate 1 (IRS1) and inhibits glucose transporter 4 (GLUT4) translocation. Conversely, binding of DGKζ to phosphatidylinositol-4-phosphate 5-kinase type-1 alpha (PIP5K1α) promotes glucose uptake. Nuclear DGKζ increases upon myogenic differentiation. Abbreviations: INSR, insulin receptor.

metabolized by distinct DGK isoforms, as well as their subcellular localization, may play an opposing role on insulin sensitivity.

Insulin and contraction/exercise increase glucose uptake in skeletal muscle by independent mechanisms [60,61]. DGKζ activates Rac1 [62], a positive regulator of GLUT4 translocation in response to insulin or exercise [63]. Of note, DGKγ negatively regulates Rac1 activity through a β2-chimaerin-dependent pathway [64], illustrating the isoform-specific effect on signal transduction. Nevertheless, the role of DGKζ in contraction/exercise-induced glucose uptake remains unknown. Upon mechanical stimulation, DGKζ-induced PA production activates mTOR [65], further supporting a role for DGKζ in mediating effects of skeletal muscle contraction on metabolism. DGKζ also plays a role in skeletal muscle differentiation and hypertrophy. In mouse skeletal muscle cells, nuclear localization of DGKζ increases during differentiation, and expression of DGKζ is necessary for complete myogenic differentiation [31]. Following functional overload, a method used to induce compensatory hypertrophy, DGKζ abundance and activity are increased [66].

Moreover, DGKζ overexpression in tibialis anterior muscle induces fiber hypertrophy [65]. Conversely, deletion of DGKζ in skeletal muscle is dispensable for functional overload-induced hypertrophy [59], suggesting that DGKζ-mediated hypertrophic response is compensable. mTORC1 activity is affected by both overexpression and deletion of DGKζ [65]. Activation of mTOR arises from external stimuli, through PI-3K/Akt signaling pathway, and intracellular stimuli. Thus, DGKζ activity modulates mTOR signaling through the local production of PA, which may influence insulin-induced metabolic processes.

Whole-body deletion of DGKζ protects against the development of high-fat-diet-induced obesity [59]. A trend for altered HSL activity and increased lipolysis in adipose tissue might account for the reduction in fat mass in this model [59]. DGKζ silencing in tumor cells reduces the abundance of sterol regulatory element-binding protein 1 (SREBP1), a transcription factor regulating genes related to glucose and fatty acid metabolism [67]. While SREBP1 reduction in adipose tissue reduces **lipogenesis** and adipogenesis, therefore limiting fat mass expansion [68], glucose utilization in other peripheral tissues may be enhanced. DGKζ knockout mice have a higher respiratory exchange ratio (RER) reflecting a preference for glucose versus lipids as an energy substrate, independent of changes in food intake and activity [59]. Moreover, reduced SREBP1 expression, via activation of Wnt/β-catenin signaling, preserves insulin sensitivity in myotubes [69]. Thus, improved insulin sensitivity in skeletal muscle of high-fat-diet-fed DGKζ knockout mice, as suggested by increased insulin-stimulated glucose uptake [59], may partly be mediated by reduced SREBP1 activity. Additionally, SREBP1 influences the expression of enzymes involved in lipid synthesis, therefore reduced activity may promote the accumulation of DAG species by reducing the utilization of DAG as triglycerides precursor. Yet SREBP1 abundance remains uncharacterized in DGKζ knockout mice. Furthermore, DGKζ expression is increased in the hypothalamus of high-fat-diet-fed rats [70], and in visceral adipose tissue of obese men with metabolic syndrome [71], suggesting that DGKζ mediates both central and peripheral effects. Collectively, these studies highlight a role for DGKζ in the development of obesity and insulin resistance, and tissue-specific contribution of DGKζ warrants further investigation.

DGKθ and Hepatic Insulin Resistance

Hepatic insulin resistance, defined by impaired suppression of **gluconeogenesis**, is characterized by increased fatty acid uptake, *de novo* lipogenesis, and very-low-density lipoprotein secretion [43]. Deletion of DGKθ in HepG2 cells induces **steatosis**, with increased total lipid content associated with decreased PA and increased DAG content [72]. These findings suggest that hepatic DGKθ regulates the balance between fatty acid storage and oxidation, most likely by regulating the availability of precursors for triacylglycerides synthesis. Knockout of DGKθ in HepG2 cells reduces phosphorylation of Akt1 and IRS1 [72], thereby attenuating insulin signaling. Phosphorylation of PKCε is increased by deletion of DGKθ [72]. Thus, DGKθ may directly induce hepatic insulin resistance by increasing DAG content and thereby affecting PKCε signaling.

Overexpression of DGKθ in primary mouse hepatocytes increases PA content and thereby reduces Akt phosphorylation [73]. Hepatic DGKθ-mediated PA production increases phosphorylation of mTOR and FoxO1, thereby repressing gluconeogenic gene expression [74]. Deletion of DGKθ increases abundance of proteins involved in gluconeogenesis and lipid synthesis such as SREBP1 and fatty acid synthase (FAS), whereas the mitochondrial enzyme carnitine palmitoyltransferase 1 (CPT1α), controlling the rate-limiting step in fatty acid β-oxidation, is decreased [72]. Reduction of CPT1α abundance is also found in embryonic fibroblasts from DGKδ knockout mice [75], but the exact mechanisms underlying the regulation of CPT1α expression by DGK remain to be determined. In liver, farnesoid X receptor (FXR), a nuclear receptor activated by bile acids involved in cholesterol and lipid metabolism, directly binds to the DGKθ

promoter, thereby increasing DGKθ transcription [74]. Furthermore, FXR activation in diabetic mouse models lowers blood glucose and lipid levels [76]. Thus, activated FXR may partly improve hepatic insulin sensitivity through increased DGKθ expression. The clinical relevance of DGKθ activation in the context of hepatic steatosis and insulin resistance remains to be determined.

DGKε and Lipid Metabolism

Elevated circulating fatty acids and aberrant fatty acid metabolism contribute to the development of insulin resistance and impaired glucose metabolism. Gene polymorphisms of the *DGKE* gene have been associated with plasma lipid levels in humans, with one SNP (c.579A>C) associated with increased triglyceride levels and decreased high-density lipoprotein cholesterol levels [77]. DAG is an activator of PKC, which induces low-density lipoprotein (LDL) oxidation and LDL-receptor expression, suggesting a role for DGKε in whole-body lipid metabolism. In high-fat-fed DGKε knockout mice, the RER is reduced [42], suggesting increased oxidation of fatty acids over carbohydrates. Expression of p53 is increased in DGKε knockout mouse embryonic fibroblasts and this induction is partially blunted by restoration of DGKε [78]. Increased DAG levels due to DGKε deletion activates PKC, which phosphorylates p38 and subsequently activates p53. Consequently, expression and activity of glycerol kinase (GK), a p53 target gene, is increased [42,78]. GK acts as a lipogenic enzyme that phosphorylates glycerol and favors its incorporation into lipid. Transcriptional regulation by p53 promotes oxidative phosphorylation while reducing glycolysis [79]. Moreover, citrate synthase protein abundance is increased in skeletal muscle of high-fat-diet-fed DGKε knockout mice, further supporting a role for DGKε in oxidative capacity [42]. Thus, p53 may be a key mediator of DGKε in facilitating fatty acid oxidation over storage by inducing the expression of genes involved in mitochondrial import and repressing SREBP1 transcription and subsequently target genes involved in lipogenesis (Figure 4). Additionally, in a separate study, high-fat-diet-fed DGKε knockout mice exhibit increased adiposity, characterized by adipocyte hypertrophy [80]. Differentiation of adipocytes is inhibited by p53 [79], and p53 expression in adipose tissue contributes to insulin resistance in high-fat-diet-induced obese mice [81]. Furthermore, increased macrophage infiltration and tumor necrosis factor (TNF)α levels are detected in adipose tissue of high-fat-fed DGKε knockout mice [80]. Aberrant adipokine and chemokine secretion may lead to macrophage recruitment to adipose tissue in obesity. Nevertheless, DGKε deletion-induced p53 expression may trigger inflammation at an early stage of obesity, as expression of proinflammatory cytokines in adipose tissue is positively regulated by p53 [81]. Ultimately, this inflammatory response contributes to the development of insulin resistance in adipose tissue and subsequent adiposity. Thus, further investigation of the contribution of DGKε following nutrient overload in adipose tissue is of interest to decipher the mechanisms involved. The tissue-specificity effects of DGKε may depend upon its subcellular location, as well as its oligomerization [82]. Despite evidence for a link between DGKε and lipid metabolism, the precise role of this isoform in metabolic regulation remains unclear.

DGK and Inflammation

DGKα is a positive regulator of immune responses and inflammation by mediating TNFα-induced activation of nuclear factor (NF)-κB [83]. Expression of interleukin (IL)-1β, a marker of macrophage activation, is reduced in white adipose tissue of DGKα knockout mice receiving a short-term high-fat diet [84], further supporting a role for DGKα in the acute inflammation response. PA produced by DGKα activates PKCζ, which in turn phosphorylates and activates NF-κB [83] to induce inflammation. However, whether nuclear DGKα plays a direct role in the transcription of proinflammatory genes remains unknown. Conversely, DGKζ deficiency increases TNFα-induced NF-κB transcriptional activity in fibroblasts [85]. Thus, DGKζ may have an opposing effect from DGKα on TNFα-induced inflammatory process. Cytoplasmic localization of DGKζ promotes p53 degradation through the ubiquitin–proteasome system while nuclear DGKζ potentiates p53

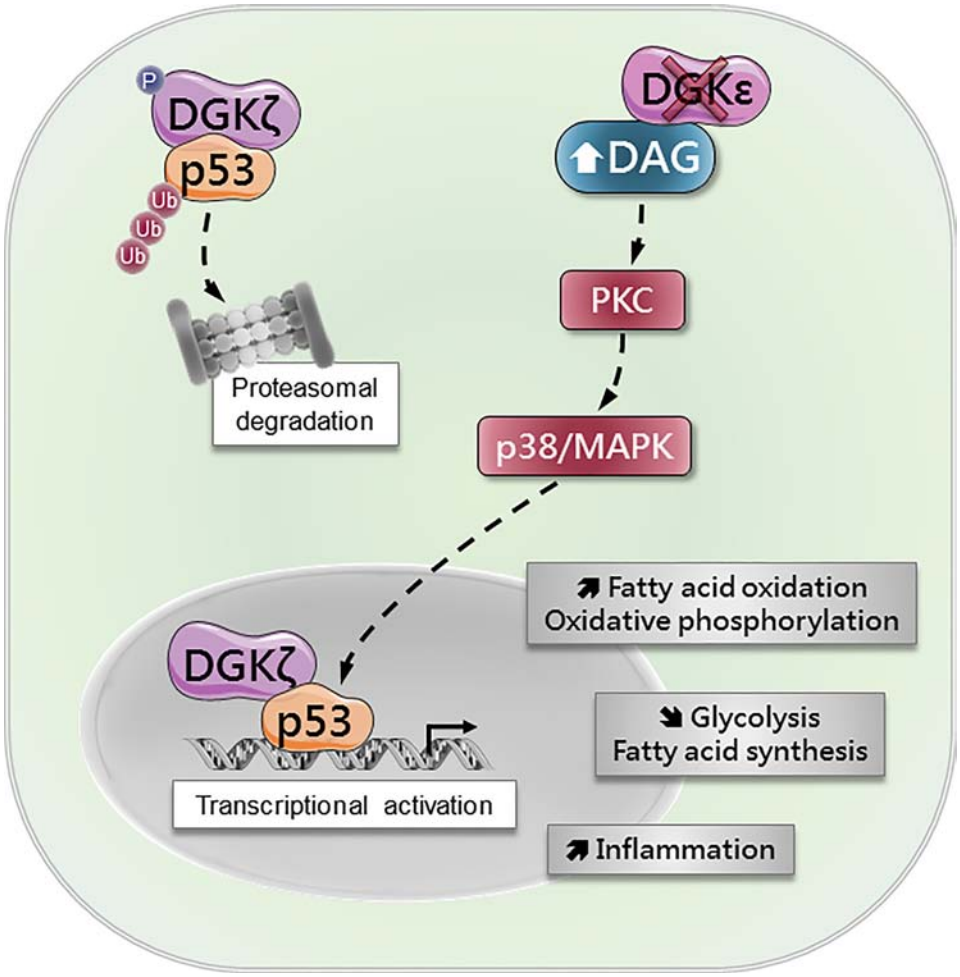

Figure 4. Role for p53 in Diacylglycerol Kinase (DGK)-Mediated Energy Homeostasis and Inflammatory Responses. DGKζ binds p53 in the cytoplasm, thereby inducing p53 proteasomal degradation. Nuclear localization of DGKζ stabilizes p53 and enhances its transcriptional activity. Increased diacylglycerol (DAG) levels in DGKε-depleted cells activates protein kinase C (PKC)–p38/mitogen-activated protein kinase (MAPK) signaling and increases p53 transcriptional activity. Activation of p53 promotes lipid oxidation and oxidative phosphorylation while reducing glycolysis and lipid synthesis. In adipose tissue and inflammatory cells, p53 induces the expression of proinflammatory cytokines.

transcriptional activity [86], whereas p53 activation increases the expression of DGKα [87] to promote inflammation. Thus, a regulatory loop between DGK isoforms may ensure cellular homeostasis under physiological conditions. However, the nature of the stimuli that regulate DGKζ subcellular localization and p53 activity remains to be determined.

Various types of cellular stress stimuli trigger an inflammatory response. Glucocorticoid treatment increases DGKη1 protein abundance [88], and oxidative stress induces the translocation of DGKη from the cytoplasm to intracellular endosomes [89]. Furthermore, lipid-induced inflammation in adipocytes increases DGKη expression, which mediates the induction of inflammatory gene expression [90]. DGKη is expressed in all insulin-sensitive tissues [28], but its role in metabolic disease remains unknown. Collectively, these results suggest that DGK isoforms have specialized roles that are dependent on tissue/organ expression profiles, subcellular localization, and interacting

partners. Whether DGK isoforms play a role in the interplay between immunological and metabolic processes and the pathogenesis of insulin resistance warrants further study.

Future Perspective: DGK, a Potential Drug Target?

DGK isoforms are implicated in many physiological and pathological processes, thus, targeting isoform-specific DGK activity is an attractive strategy for the treatment of inflammatory or metabolic diseases. Classical strategies in basic research include overexpression, deletion, or silencing, but these approaches have limitations in a clinical setting and thus, compounds specifically targeting DGK activity may be a useful tool to decipher the precise role of each DGK isoform. To date, there are two commercially available inhibitors of DGK enzymes, R59022 [91] and R59949 [92]. These inhibitors are not fully specific to DGK enzymes and inhibit the activities of different isoforms [93]. Development of new isoform-specific activating or inhibiting compounds is therefore needed to dissect the role of these enzymes in metabolism.

Due to its role in cancer, development of a DGKα-specific inhibitor has received more attention than other isoforms over the last years. Recently, a chemical compound library screening approach identified a DGKα-specific inhibitor, CU-3, which inhibits its activity by competing with ATP [94]. Ritanserin, a potential inhibitor selected due to its similar chemical structure to R59022, also exhibits an inhibitory effect on DGKα, but with higher potency than CU-3 (IC50 of 15 μM versus 0.6 μM, respectively) [95]. The ATP-binding site of DGK isoforms has been recently characterized by activity-based protein profiling [96]. Further characterization led to the discovery of a ritanserin-derived fragment exhibiting higher specificity for DGKα against the whole kinome [96]. Similar studies designed to characterize binding regions may identify new compounds that modulate the activity of specific DGK isoforms. This may facilitate progresses in understanding DGK isoform-specific biological functions and uncover novel pharmacological strategies to target DGK isoforms for metabolic diseases.

Concluding Remarks

Animal models have provided useful tools to understand the contribution of each DGK isoform to whole-body homeostasis. To date, several whole-body knockout models have been generated and each isoform has been shown to play an important role in energy homeostasis. Whereas the contribution of specific DGK isoforms to the regulation of energy homeostasis and insulin action is emerging, the underlying molecular mechanisms remain unclear (see Outstanding Questions). Decreased DGKδ activity leads to the development of insulin resistance and hepatic deletion of DGKθ induces steatosis and associated insulin resistance, concomitant with increased DAG levels in both cases. However, accumulation of DAG in insulin-sensitive tissues is not always linked to the development of insulin resistance. Deletion of DGKε or DGKζ improves skeletal muscle insulin sensitivity and whole-body energy homeostasis, despite elevations in total DAG content. Hence, distinct DGK isoforms metabolize specific DAG species at precise subcellular localization and play opposing roles on insulin resistance. In addition to the control of cellular DAG content, DGK isoforms also exert effects through direct binding to specific partners. Therefore, the development of tissue-specific overexpression, knockout, or inducible models will allow a better understanding of the contribution of each isoform to the pathogenesis of metabolic disease, and validate the utility of compounds that modulate DGK activity to improve glucose and energy homeostasis.

Outstanding Questions

What is the tissue-specific contribution of each DGK isoform to the pathogenesis of metabolic disease?

Do specific intracellular pools of DAG species promote insulin resistance?

What are the specific DGK isoform-binding partners?

Is p53 a key transcriptional mediator of DGK regulation of lipid and glucose metabolism?

Are DGK isoforms pharmacological targets for improving insulin sensitivity in human?

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