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Review Article

Phospholipase C-related catalytically inactive protein can regulate obesity, a state of peripheral inflammation

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Summary Obesity is defined as abnormal or excessive fat accumulation. Chronic inflammation in fat influences the development of obesity-related diseases. Many reports state that obesity increases the risk of morbidity in many diseases, including hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, sleep apnea, and breast, prostate and colon cancers, leading to increased mortality. Obesity is also associated with chronic neuropathologic conditions such as depression and Alzheimer's disease. However, there is strong evidence that weight loss reduces these risks, by limiting blood pressure and improving levels of serum triglycerides, total cholesterol, low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol. Prevention and control of obesity is complex, and requires a multifaceted approach. The elucidation of molecular mechanisms driving fat metabolism (adipogenesis and lipolysis) aims at developing clinical treatments to control obesity. We recently reported a new regulatory mechanism in fat metabolism: a protein phosphatase binding protein, phospholipase C-related catalytically inactive protein (PRIP), regulates lipolysis in white adipocytes and heat production in brown adipocytes *via* phosphoregulation. Deficiency of PRIP in mice led to reduced fat accumulation and increased energy expenditure, resulting in a lean phenotype. Here, we evaluate PRIP as a new therapeutic target for the control of obesity.

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Contents

1. Introduction

The World Health Organization points out that obesity is currently one of the most blatantly visible, yet most neglected, public health problem. If immediate action is not taken, millions will suffer from an array of serious health disorders linked to obesity [1]. Recent studies have demonstrated that obesity is associated with some of the cellular mechanisms of inflammation and overproduction of proinflammatory cytokines. The pathological conditions of adipose tissue under hypertrophy (cell size increase) and hyperplasia (cell number increase) are low-grade and chronic inflammation may lead to the development of various co-morbidities, such as hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, and cancers [2,3].

Inflammation is a physiological response necessary to restore homeostasis altered by diverse stimuli; however, an excessive inflammatory response or a chronically established inflammation state can cause systemic deleterious effects. For instance, periodontal disease is a source of chronic inflammation and was found to play a causative or contributory role in the pathogenesis of systemic diseases [4]. Furthermore, epidemiological studies have recently shown high co-morbidity between mental illness and peripheral chronic inflammatory diseases, including obesity and periodontitis [2—4]. Therefore, the control of inflammation in peripheral organ diseases is paramount for the protection and promotion of human health.

Adipose tissues are an insidious source of inflammation in morbid obesity. We have elucidated a new regulatory mechanism in lipid and energy metabolisms within adipocytes to control obesity. Phospholipase C-related catalytically inactive protein (PRIP), a new functional molecule in lipolysis, is involved in a triacylglycerol (TAG) degradation pathway *via* intracellular phosphoregulation. Here, we discuss the regulation of TAG hydrolysis *via* PRIP.

2. Fat metabolism in adipocytes

2.1. Regulation of fat metabolism

There are two types of adipose tissues, white adipose tissue (WAT) and brown adipose tissue (BAT), which have antagonistic functions [5]. WAT is the major energy storage component in higher eukaryotes. The primary purposes of this tissue are synthesis and storage of TAG in periods of energy excess, and hydrolysis of TAG to generate fatty acids for use by other organs during periods of energy deprivation. In contrast, BAT directly dissipates the chemical energy contained in fatty acids as heat *via* uncoupling protein 1 (UCP1) as a defense against cold and excessive feeding (Fig. 1). Understanding of molecular mechanisms driving adipocyte-specific TAG synthesis and hydrolysis, and/or governing energy expenditure in brown adipocytes is critical to explain the development of obesity.

2.2. Activation of lipolysis by catecholamine signaling

Although TAG synthesis occurs in multiple tissues, TAG lipolysis during periods of energy demand predominantly occurs in adipose tissues. The hydrolytic action by lipases in WAT is rapid, and free fatty acids (FFAs) are supplied to other organs to meet the energy requirements of the organism [6]. The TAG degradation process is regulated by several molecules, including anti-comparative gene identification 58 (CGI-58), perilipin (lipid droplet-associated protein), and the lipolytic enzymes, adipocyte triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL). These lipolytic enzymes and modulators promote the hydrolysis of TAG, resulting in the formation of FFAs and glycerol in adipocytes (Fig. 1).

The intracellular degradation of TAG is catalyzed by a cascade of lipolytic enzymes (Fig. 2). Under basal condition, at the surface of lipid droplets, perilipin, a master lipolysis regulator of stored TAG, sequesters CGI-58, a coactivator protein of ATGL [7]. This prevents sequential hydrolysis of stored TAG. Under starvation conditions, sympathetic nerves are activated, which initiates adipose lipolysis. Catabolic hormone adrenaline, a catecholamine, binds to β-adrenergic receptors on adipocyte plasma membrane and triggers a G protein-mediated cascade that activates adenylate cyclase, which itself increases levels of cAMP and activates protein kinase A (PKA). PKA phosphorylates perilipin at multiple sites. Subsequently, CGI-58 is released from perilipin, associates with, and fully activates ATGL.

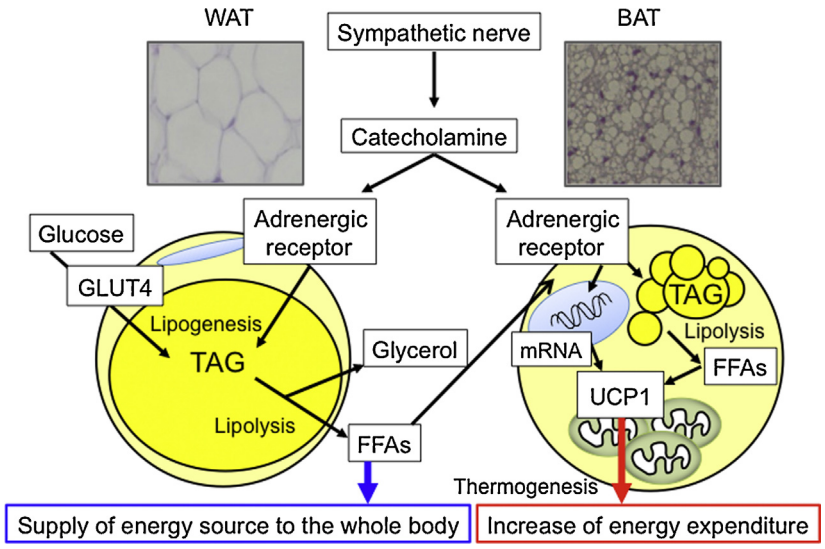

Figure 1 Fat metabolism in adipocytes. WAT is the primary energy storage depot, accumulating fuel reserves in the form of TAG during times of energy excess, and releasing FFAs and glycerol during periods of energy deprivation. BAT is specialized in dissipating chemical energy in the form of heat *via* UCP1, which is activated by sympathetic nerve stimulation. WAT, white adipose tissue; BAT, brown adipose tissue; TAG, triacylglycerol; FFA, free fatty acid; UCP1, uncoupling protein 1; GLUT4, glucose transporter 4.

This ATGL hydrolyzes TAG into diacylglycerol (DAG). The activated PKA also phosphorylates HSL, located in the cytosol. Phosphorylated HSL is translocated from the cytosol to the surfaces of intracellular lipid droplets and is tethered on the lipid droplets by the phosphorylated perilipin. Subsequently, activated (phosphorylated) HSL hydrolyzes DAG into monoacylglycerol, followed by degradation into a FFA and glycerol by MGL in the final step of lipolysis. FFAs are released into the blood stream, where they bind to albumin for transport to surrounding tissues requiring energy. Glycerol is also transported into the bloodstream and is absorbed by the liver or kidneys, where it is converted to glycerol 3-phosphate by the enzyme glycerol kinase.

Perilipin is phosphorylated at multiple sites by PKA, a process essential for the translocation of HSL to lipid droplets. Furthermore, this response corresponds to the phosphorylation of HSL and the acceleration of lipolysis promoted by the sympathetic nervous system in adipocytes. HSL can be phosphorylated at least on five serine residues (563, 565, 600, 659, and 660 of the rat sequence) *in vitro* [7]. Ser-563, Ser-659, and Ser-660 are the major PKA phosphorylation sites responsible for activating HSL. Dephosphorylation of HSL and perilipin by protein phosphatases can also play an important role in the regulation of lipolysis.

2.3. Inhibition of lipolysis by insulin receptor signaling

Insulin, the major anabolic hormone, can restrain lipolysis and promote fat storage in adipose tissues during the postprandial period. Protein phosphatases are involved in the antilipolytic effect of insulin. Insulin receptor tyrosine kinase phosphorylates insulin receptor substrate (IRS)-1, which recruits and activates phosphoinositide 3-kinase (PI3K) leading to the production of phosphatidylinositol-3,4,5-triphosphate (PIP_3). AKT (also known as protein kinase B) is recruited onto the membrane by binding with PIP_3 *via* its pleckstrin homology domain, and is subsequently phosphorylated and activated by PDK1. AKT thus phosphorylates two important targets, phosphodiesterase 3B [8] and protein phosphatases (protein phosphatase 1, protein phosphatase 2A, and protein phosphatase 2C) [9,10], to allow the down-regulation of lipolysis. Consequently, lipolysis in adipocytes is attenuated. In addition, insulin signaling-independent dephosphorylation of HSL and perilipin may be involved in the inactivation of lipolysis after activation of the sympathetic nervous system; however, this mechanism has not yet been elucidated.

3. A new molecule for the regulation of lipolysis in adipocytes

3.1. Identification of PRIP

PRIP was originally purified as an inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] binding protein from the cytosol fraction of the rat brain, but has since been isolated from the membrane fraction of the brain [11,12]. PRIP has similar domain organization to phospholipase C-δ1 [13,14], but lacks the enzymatic activity of phospholipase C [15] (Fig. 3). There are two isoforms in mammals; PRIP1, expressed mainly in the brain, and PRIP2, ubiquitously expressed [16,17]. Both PRIPs are expressed in white and brown adipose tissues [18,19]. Experiments exploring a PRIP binding partner revealed that PRIP can bind to PP1 and PP2A [20–25], Ins(1,4,5)P_3 and inositol lipids [11,12,26–28], gamma aminobutyric acid type A receptor-associated protein (GABARAP) [29–33], β subunit of $GABA_A$ receptor [21], and phosphorylated AKT [34]. After verifying the relationship between PRIP and these binding partners using *Prip*-KO mice, we elucidated the role of PRIP in cells; PRIP is involved in Ins(1,4,5)P_3/Ca^{2+} signaling [35,36], dephosphoregulation of

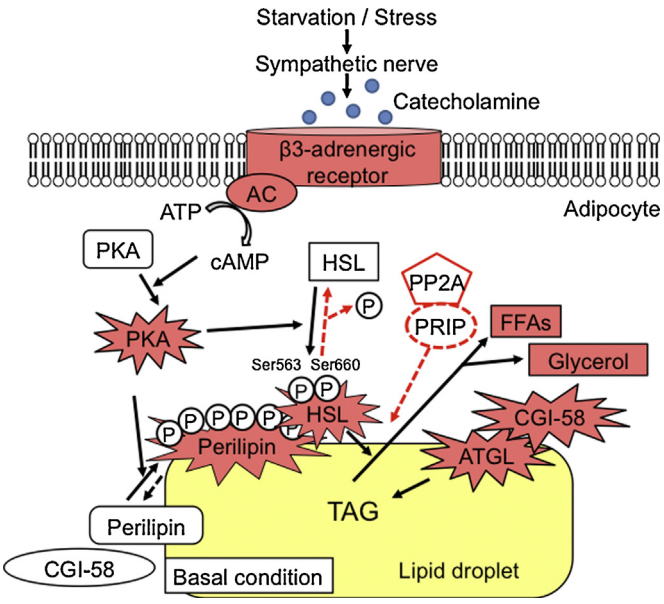

Figure 2 Possible mechanism by which the PRIP and protein-phosphatase complex mediates lipolysis regulation. Lipolysis in adipocytes is mediated by the activation of a PKA-mediated pathway. The process is regulated by lipases (HSL and ATGL) and other modulatory proteins, including perilipin, CGI-58, PP2A, and PRIP. Phosphorylation of perilipin releases CGI-58, resulting in the activation of ATGL by association with CGI-58. Phosphorylated HSL is translocated to the lipid membrane and degrades TAG and/or DAG. These events are also regulated by phosphatases (PP1 and PP2A), whose activities are modulated by PRIP. The disappearance of the dotted line represents postulated situations in *Prip*-DKO mice. AC, adenylate cyclase; ATGL, adipocyte triglyceride lipase; TAG, triacylglycerol; FFA, free fatty acid; P, phosphate group; PKA, protein kinase A; HSL, hormone-sensitive lipase; PRIP, phospholipase C-related catalytically inactive protein; PP2A, protein phosphatase 2A; CGI-58, comparative gene identification 58 (abhydrolase domain-containing protein 5).

intracellular signaling molecules [18,19,21,22], intracellular trafficking of $GABA_A$ receptors or insulin secretory vesicles [29,30,33,37–40], and regulation of autophagy [31,32].

3.2. Lean phenotype of *Prip*-KO mice

Studies in *Prip*-null knockout (*Prip*-KO) mice have confirmed the importance of this molecule in the lipolysis of TAG [18,19]. Regular diet (RD)-fed *Prip*-KO mice exhibited a lean phenotype with smaller WAT in size and weight. The *Prip*-KO mice showed slightly more food intake than wild-type mice. The serum cholesterol and TAG levels were significantly increased in *Prip*-KO mice compared with wild-type control mice. However, ectopic lipid accumulation was not observed in the liver of *Prip*-KO mice. In high-fat diet (HFD)-feeding experiments, *Prip*-KO mice showed less body weight increase, and their body weight at 20 weeks of age was lower than that of wild-type controls. The *Prip*-KO mice obviously displayed leanness with small-sized WAT. These results allowed associating the lean phenotype of *Prip*-KO mice with the alteration of fat metabolism in adipocytes.

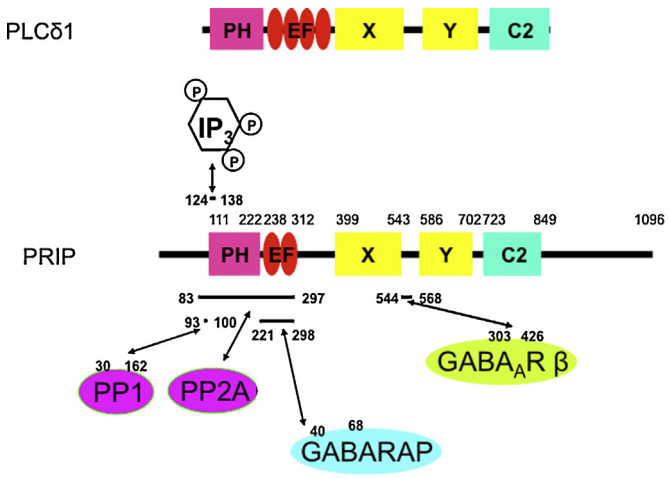

Figure 3 Phospholipase C-related catalytically inactive protein. PRIP has a similar domain organization to PLCδ1. The binding partners of PRIP are shown. The numbers represent amino acid residues. IP_3, inositol 1,4,5-trisphosphate; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; GABARAP, $GABA_A$ receptor-associated protein; $GABA_AR$ β, $GABA_A$ receptor β subunit; PH, pleckstrin homology domain; EF, EF hand domain; X and Y, catalytic subunit of PLC; C2, C2 domain.

3.3. PRIP and protein phosphatase regulate adrenaline-induced lipolysis in adipocytes

Our investigations showed that phosphorylation of HSL and perilipin in RD-fed *Prip*-KO WAT was upregulated under both non-fasting and fasting conditions compared with wild-type mice. In response to adrenaline stimulation, or under starvation conditions, the cytosolic protein PRIP was translocated to lipid droplets in mouse white adipocytes; in the meantime, levels of PP1 and PP2A, which bind to PRIP, were increased in the lipid droplet fractions (Fig. 2). Consistently, after adrenaline stimulation, time-dependent dephosphorylation change of HSL was observed in wild-type adipocytes, but not in *Prip*-KO adipocytes. From these findings, we proposed a model of PRIP-mediated phosphoregulation of lipolysis in adipocytes (Fig. 2). Once starvation or stress signals trigger the activation of PKA in adipocytes, HSL is phosphorylated, translocated to lipid droplets, and activates hydrolysis of lipids. The signal also induces the translocation of PRIP and protein phosphatase complex to lipid droplets, which promotes the dephosphorylation of HSL and attenuates lipolysis. These sequential events yield a sharp transient activation of lipolysis to provide a fine-tuning of catabolic hormonal regulation in adipocytes.

3.4. PRIP regulates energy metabolism

Compared to wild-type mice, HFD-fed *Prip*-KO mice showed more moderate body weight increase, greater glucose tolerance, and higher insulin sensitivity. This underlined a protection mechanism against HFD-induced obesity in *Prip*-KO mice [19]. Histological analyses showed that ectopic lipid accumulation in the liver was strongly decreased in HFD-fed *Prip*-KO mice. Consistently, energy expenditure and

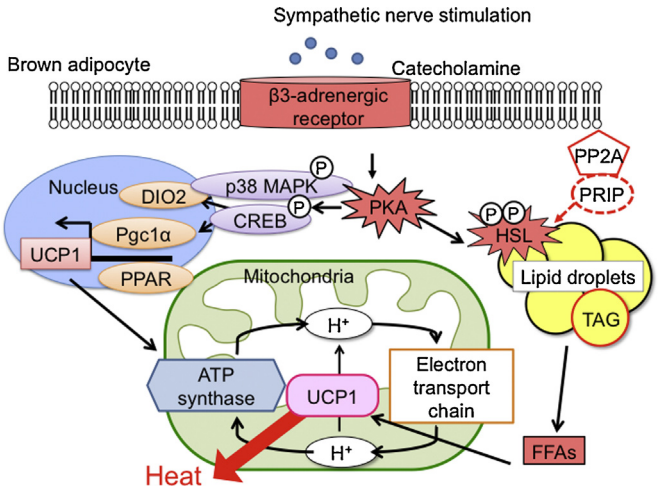

Figure 4 PRIP regulates thermogenesis in brown adipocytes. Sympathetic nerve stimulation enhances receptor-mediated PKA activation in brown adipocytes. PKA induces UCP1 activation through at least two pathways. (i) PKA facilitates the phosphorylation of HSL and perilipin, which promotes lipolysis. The resulting FFAs enhance UCP1 enzymatic activation and *Ucp1* gene expression through PPAR-mediated PGC1α activation (the pathway represented by the solid arrows). (ii) PKA also induces phosphorylation of CREB and p38 MAPK, which activate PGC1α and DIO2. Consequently, *Ucp1* gene expression and activity are elevated. *Prip* gene ablation (dotted line) leads to elevation of HSL phosphorylation-mediated lipolytic cascades followed by FFA-mediated UCP1-induced heat production. CREB, cAMP response element-binding protein; DIO2, type II iodothyronine 5′-deiodinase; Encircled Ps, phosphoproteins; FFA, free fatty acid; HSL, hormone-sensitive lipase; PKA, cAMP-dependent protein kinase; p38-MAPK, p38 mitogen-activated protein kinase; PGC1α, peroxisome proliferator-activated receptor γ co-activator 1α; PPase, protein phosphatase; PPAR, peroxisome proliferator-activated receptor; PRIP, phospholipase C-related catalytically inactive protein; TAG, triglyceride; UCP1, uncoupling protein 1.

body temperature were higher in *Prip*-KO mice than in wild-type mice.

Many studies have shown that BAT contributes to adult human obesity, and activation of UCP1-mediated thermogenesis in BAT prevents obesity and diabetes [5,41,42]. Brown adipocytes directly dissipate the chemical energy in fatty acids as heat through uncoupling protein 1 (UCP1) (Fig. 4). In both white and brown adipocytes, sympathetic hyperactivity causes β-adrenergic receptor-induced PKA activation, followed by activation of lipolysis. FFAs are used not only as substrates for oxidative respiration but also as allosteric activators of UCP1. In addition, PKA activation followed by elevated intracellular FFAs activates UCP1-mediated heat generation and regulates thermogenesis through transcriptional control in BAT [19,43–45]. PKA mediates the phosphorylation of cAMP response element-binding protein (CREB) and p38 mitogen-activated protein kinase (p38 MAPK), followed by activation of the gene expression of peroxisome proliferator-activated receptor (PPAR)-γ, coactivator-1α (PGC1α), PPARα, and PPARδ, to finally trigger *Ucp1* gene expression in brown adipocytes [5,45–47]. The *Prip*-KO mouse study demonstrated that PRIP regulates β-adrenergic receptor signaling-induced UCP1-dependent thermogenesis in BAT through phosphoregulation of HSL and perilipin.

4. Chronic peripheral inflammation is a risk factor for mental illness

Obesity is a chronic inflammatory state that originates locally in adipose tissues as a consequence of excessive fat deposition, and is later reflected in increased systemic circulating levels of proinflammatory proteins. Markers of abdominal obesity (*e.g.*, waist circumference) seem to be strongly associated with inflammatory markers, and a reduction in the adipose tissue mass reduces the ability of adipose tissues to produce proinflammatory cytokines, TNFα, IL-6, IL-8, and leptin [48–50]. Dental diseases such as periodontitis are also associated with high levels of systemic inflammation [51], a significant predictor of inflammatory illnesses, such as cardiovascular disease [52,53] and diabetes [54]. Moreover, many studies show that peripheral chronic inflammation increases the risk of mental illness [3,4]. Depressive patients have high concentration of proinflammatory cytokines (*i.e.*, IL-1β, IL-6, and TNF-α) in blood [55].

In animal models using rodents, systemic inflammation induces ''depression-like behavior'' such as anhedonia, reduced appetite, helplessness, apathy, and social withdrawal [56]. We and other groups showed that peripheral injection of lipopolysaccharide induced anorexia *via* the increase of hypothalamic IL-1β and IL-6 [57,58]. The circulating proinflammatory cytokines can enter the central nervous system in areas where the blood–brain barrier is incomplete, or can be transported into the brain tissues by carrier-mediated mechanisms across the blood–brain barrier. Therefore, proinflammatory cytokines in peripheral blood stream communicate with the brain and cause the activation of microglia and astrocytes. In the case of microglia, TNF-α activated indoleamine 2,3-dioxygenase, an enzyme enabling the production of kynurenine from tryptophan, the origin of serotonin [57]. This resulted in decreasing the serotonin synthesis pathway and promoting kynurenine production, itself further metabolized into quinolinic acid. Quinolinic acid is an *N*-methyl-D-aspartate (NMDA) receptor agonist, which induces depression-like behavior. Therefore, peripheral inflammation, mediated by obesity or oral infectious diseases, induces the decrease of serotonin and the increase of neurotoxic quinolinic acid, potentially causing mental illness. Therefore, the control of peripheral inflammation is crucial for maintaining a healthy life.

5. Conclusion

We recently defined PRIP as a new modulator within the lipolysis pathway, which negatively regulates the phosphorylation of perilipin and HSL in WAT, and can also regulate thermogenesis in BAT *via* fatty acid production. Importantly, deficiency of PRIP in mice exhibited anti-obesity phenotypes. Therefore, PRIP represents a potential therapeutic target for the control of obesity. Since anti-obesity therapy can reduce the risks of many serious health disorders

including hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, and mental disorders, further comprehensive studies of PRIP signaling will contribute to the development of new therapeutic targets aimed at tackling excess body fat accumulation to ensure a healthy life.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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