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Review

APPLs: More than just adiponectin receptor binding proteins

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ABSTRACT

APPLs (adaptor proteins containing the pleckstrin homology domain, phosphotyrosine binding domain and leucine zipper motif) are multifunctional adaptor proteins that bind to various membrane receptors, nuclear factors and signaling proteins to regulate many biological activities and processes, such as cell proliferation, chromatin remodeling, endosomal trafficking, cell survival, cell metabolism and apoptosis. APPL1, one of the APPL isoforms, was the first identified protein and interacts directly with adiponectin receptors to mediate adiponectin signaling to enhance lipid oxidation and glucose uptake. APPLs also act on insulin signaling pathways and are important mediators of insulin sensitization. Based on recent findings, this review highlights the critical roles of APPLs, particularly APPL1 and its isoform partner APPL2, in mediating adiponectin, insulin, endosomal trafficking and other signaling pathways. A deep understanding of APPLs and their related signaling pathways may potentially lead to therapeutic and interventional treatments for obesity, diabetes, cancer and neurodegenerative diseases.

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1. Introduction

An APPL (adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain and leucine zipper motif) was first identified as an AKT2-interacting protein using yeast two-hybrid screening in 1999 and was named after its unique structure [1]. As one of the APPL isoforms, APPL1 was first called DIP-13α (DCC-interacting protein 13α) due to its ability to interact with the tumor suppressor protein DCC (deleted in colorectal cancer), playing an important role in DCC-induced apoptosis [2]. DIP-13β, an isoform of DIP-13α, was accordingly named as APPL2 [3].

APPL1 is widely expressed in many cells and tissues. High APPL1 content was identified in differentiated C2C12 and L6 myoblast cells and in INS1 insulinoma cells [4]. Moderate expression levels of APPL1 were observed in HEK293 cells, hepatocytes and non-differentiated C2C12 myoblasts [4] as well as HeLa cells [4], MiaPaCa2 cells and Capan-1 pancreatic carcinoma cells [5]. In rodent tissues, high expression levels of APPL1 were reported in the mouse brain, skeletal muscle, fat, liver, heart, and spleen and, to a lesser extent, the pancreas and kidney [4]. In humans, high expression levels of APPL1 were detected in fat, the liver, muscle, brain, and, in particular, the pancreas [6,7]. Similarly, moderate expression levels of APPL2 were observed in fat and the brain [8], and high expression levels were observed in the kidney and pancreas tissues [8].

As an adaptor protein, APPL1 is essential for insulin signaling pathways and plays a vital role in the insulin-sensitizing effect of adiponectin [4]. APPL1 also regulates many biological activities and processes, including cell proliferation, chromatin remodeling [3], endosomal trafficking [9], cell survival [10], cell metabolism and apoptosis [2]. It interacts and synergizes with Dvl2 to regulate activating protein1 (AP-1)-dependent transcription in non-canonical Wnt signaling [11]. Apart from these signaling pathways, APPL1 also plays a role in animal development and acts on the reproductive system [12]. Dysregulation of APPL1 is involved in the pathogenesis of some diseases, including familial diabetes, Down syndrome and Alzheimer's disease. Moreover, APPL1 may be involved in diabetic complications as patients with atherosclerotic plaques of type 2 diabetes (T2D) had lower APPL1 levels compared to non-diabetic patients [13]. Hence, it is important to explore the roles and the regulation of APPL1 for the development of new strategies against metabolic syndrome, T2D and related cardiovascular disease, Down syndrome and Alzheimer's disease. In this review, we summarize recent discoveries on the functions of APPLs, particularly APPL1, in adiponectin, insulin, endosomal trafficking and other signaling pathways.

2. Structure of APPLs

2.1. Structure of APPL1

APPLs are highly conserved in vertebrates. Homologous sequences of both APPL1 and APPL2 have been found in all vertebrate genomes, including humans and zebrafish [3]. In humans, APPL1 is 710 amino acids in length and is characterized by five functional domains [14]. Briefly, APPL1 consists of the N-terminal Bin1/amphiphysin/rvs167 (BAR) domain (17–268 amino acids initially identified as the leucine zipper motif), followed by a pleckstrin homology (PH) domain (278–374 amino acids), a BPP (region between PH and PTB domains) domain (375–499 amino acids), a phosphotyrosine binding (PTB) domain (500–625 amino acids), and a CC domain at the C- terminus [3,14] (Fig. 1). For a detailed crystal structure of APPL1, please see Li et al. [14].

All five domains of APPL1 can bind to lipids, but each domain has a unique binding affinity that contributes to the interaction of APPL1 with various signaling proteins [15]. Of note, the BAR, PH and PTB domains are major functional domains. Reptin, a transcriptional repressor that binds to β-catenin and HDAC1 (histone deacetylase 1), interacts with the PH domain of APPL1 to regulate Wnt signaling [16]. The BAR and PH domains of APPL1 usually act as a single unit to mediate membrane interactions [3,14,17]. They also bind to Rab 5 [3,14,17], a small GTPase involved in endosomal trafficking [17].

The PTB domain is close to the C terminus, ensuring an easy binding structure for its binding partners [18]. This domain interacts with a diverse set of receptors, including netrin-1 receptor DCC [2], nerve growth factor (NGF) receptor TrkA [9,19], follicle-stimulating hormone (FSH) receptor (FSHR) [20,21], epidermal growth factor (EGF) receptor (EGFR) [22], and adiponectin receptors AdipoR1 and AdipoR2 [4,23]. The PTB domain also interacts with signaling proteins, such as AKT [1,21,24], PI3K subunits [1,25], βCTF [7], and TRAF2 [26], demonstrating its importance in various signaling pathways.

The BPP domain between the PH and PTB domains interacts with oculocerebrorenal syndrome of Lowe (OCRL) and inositolpolyphosphate-5-phosphatase (INPP5B), both of which are the key regulators in the endocytic pathway [27].

Phosphorylation is important for the activation of APPL1, and there are 13 phosphorylation sites in the APPL1 sequence. Among them, four sites are found within the APPL1 interacting domains [28]; specifically, serines 97/98 (Ser97/98) are located in the BAR domain, Ser374 and Tyr378 are clustered near the edge of the PH domain and tyrosine 604 is in the PTB domain [28] (Fig. 1). Eight putative kinase sites have been identified at the APPL1 phosphorylation sites, including Ser401, Ser427, Ser430, Ser491, Ser689, Ser691, Ser693 and Ser696 [28]. Among them, Ser401 [29] and Ser430 [28] are particularly important because they mediate the crosstalk between the insulin and adiponectin pathways. Moreover, phosphorylation of APPL1 at Ser401 is rapidly stimulated by insulin, thus mediating the insulin-regulated binding of APPL1 with the insulin receptor β (IRβ) and dissociation with the insulin receptor substrate (IRS) in muscle cells [29]. The serine/threonine kinase glycogen synthase kinase 3 (GSK3) is a downstream effector of AKT that phosphorylates APPL1 at Ser401 [28]. Phosphorylation of APPL1 at Ser430 is augmented in cultured mouse hepatocytes with endoplasmic reticulum (ER) stress [30], suggesting that it may take part in ER stress-induced insulin resistance. Dong and colleagues found that overexpression of an APPL1 Ser430 mutant impaired the effect of APPL1 on insulin-stimulated AKT phosphorylation at Thr308, showing the negative regulatory role of APPL1 phosphorylation in insulin signaling [30]. Recently, Wang et al. (2016) identified Ser707 as a novel phosphorylation site at the C-terminus that was critical for the binding of APPL1 to postsynaptic density protein 95 (PSD95) and for the activation of the AKT signaling pathway during synaptic activity [31,32]. This phosphorylation of APPL1 at Ser707 might regulate the neuroprotective AKT signaling pathway in *N*-methyl-D-aspartate receptor (NMDAR)-dependent synaptic activation [31,32].

2.2. Structure of APPL2

As an isoform of APPL1, APPL2 shares a high level of amino acid sequence homology (~54% similarity) and identical functional domains with APPL1 [33]. Similar to APPL1, APPL2 consists of an N-terminal BAR domain, a central PH domain, and a C-terminal PTB domain [8]. Despite these similarities, structural analyses reveal that APPL2 acts in the form of two homodimers in comparison to a single homodimer form for APPL1 [34].

2.3. Genetic variations of APPLs and diseases

Accumulated evidence shows that genetic variations and mutations of APPLs are associated with a variety of human diseases. In T2D patients, single nucleotide polymorphisms (e.g., rs3806622 and rs4640525) in APPL1 are linked to abnormal body fat distribution [35]. Genetic variations (e.g., rs2272495) in APPL2 are associated with overweight and obesity in people with normal glucose tolerance [36]. In addition, genetic variations in APPL1 (e.g., rs4640525) or APPL2 (e.g., rs11112412) are associated with increased risk of coronary artery

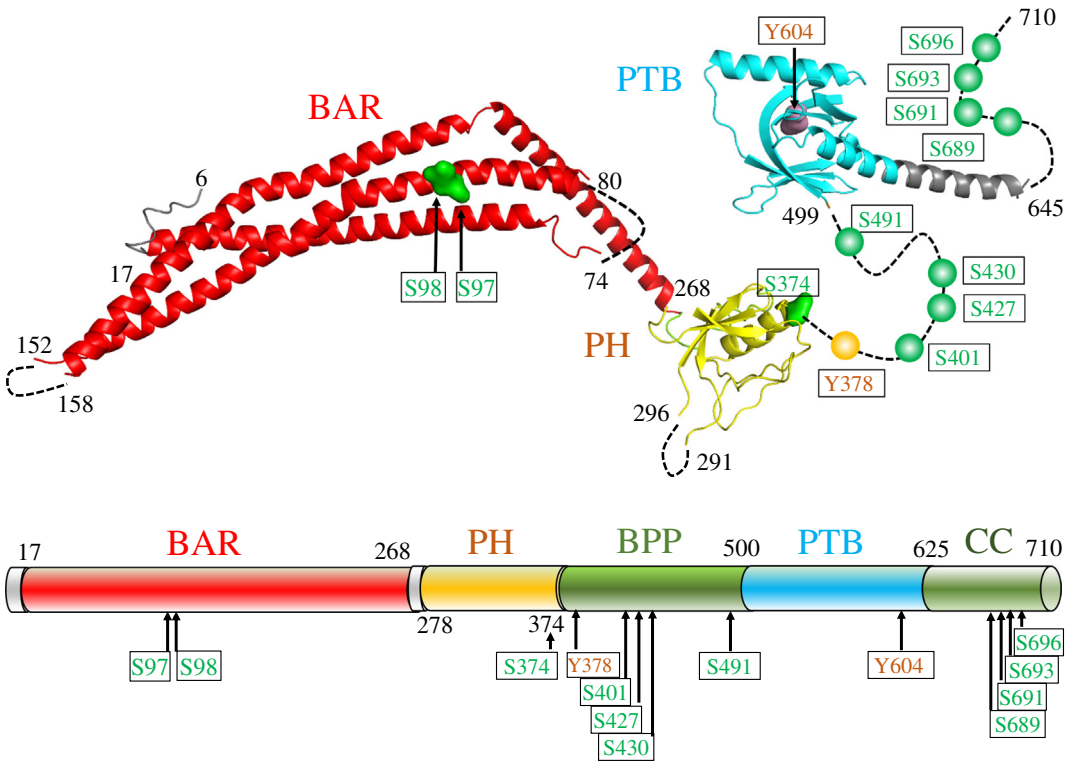

Fig. 1. Structural representation of the APPL1 protein. Cartoon representation of the APPL1 protein structure is shown on the top. Three functional domains are colored by red (BAR), yellow (PH) and blue (PTB). Green and orange spheres indicate the serine and tyrosine residues, respectively, that are exposed to phosphorylation. As the APPL1 structure is partially crystalized, we use dashed lines to indicate the structural domains that have not yet been crystallized. For visualization purposes, the PDB data (2ELA and 2ELB) were extracted from the RCSB Protein Data Bank (http://www.rcsb.org/). The visualization software used was PyMOL V1.7 (http://www.pymol.org/). A schematic view of the APPL1 functional domains and phosphorylation sites is shown beneath the protein structure. BAR, Bin/amphiphysin/Rvs domain; PH, pleckstrin homology domain; BPP, region between PH and PTB domains; and PTB, phosphotyrosine binding domain.

disease in T2D [37]. Furthermore, the combination of C-APPL1/A-APPL2 alleles is correlated with the occurrence of non-alcoholic fatty liver disease (NAFLD) with a more severe hepatic steatosis grade and a reduced adiponectin cytoprotective effect in the liver [38]. Recently, it has been reported that the GG genotype and the G carrier (CG + GG) genotypes of the rs4640525 polymorphism in the APPL1 gene were potential biomarkers for NAFLD susceptibility [39]. APPL1 mutations (c.1655 T > A [p.Leu552*] and c.280G > A [p.Asp94Asn]) are related to familial forms of diabetes [40]. Overall, these examples suggest a key regulatory role of APPL1 in glucose/lipid homeostasis, indicating that APPL1 may serve as a potential pharmaceutical target for the treatment of diabetes and related diseases.

3. APPL1 and APPL2: friends or foes?

3.1. APPLs in cellular proliferation

Since APPL1 and APPL2 share high sequence homology and many identical binding partners, it is not surprising that they are both involved in the same signaling pathways to regulate cell proliferation and apoptosis. In response to extracellular stimulation via the interaction with small GTPase Rab 5, APPL1 and APPL2 bind to components of the nucleosome remodeling and histone deacetylase complex (NuRD/MeCP1). This process mediates signaling between the plasma membrane and the nucleus, which is necessary for cell cycle progression and development [3]. Knockdown of either APPL1 or APPL2 was unable to elicit a specific cell cycle arrest, suggesting that they are both essential for cellular proliferation [3]. In addition, APPL1 and APPL2 have been shown to modulate DNA repair and to promote cell survival after radiation in pancreatic cancer cells [5]. In MiaPaCa2 and Capan-1 cells, depletion of APPLs dramatically enhanced radiosensitivity whereas the overexpression of APPLs resulted in enhanced radioresistance [5].

3.2. APPLs in apoptosis and development

Both APPL1 and APPL2 are critical for apoptosis. Studies using zebrafish models showed that either APPL1 or APPL2 knockdown caused apoptosis in fish tissues, and overexpression of APPL1 promoted cell survival as APPL1 selectively regulates apoptosis by controlling AKT signaling [10]. Similarly, in the frog *Xenopus laevis*, APPL1 knockdown led to a gut-coiling defect, strong apoptosis in the endodermal organs, pancreatic and stomach/duodenal hypoplasia, or even aplasia, during tadpole development [41]. These findings suggest that APPLs are important for the development of fish and frogs.

Conversely, studies in rodents found that APPL1 and APPL2 might be dispensable mouse development. Similar to APPL1 (Appl1 −/−) or APPL2 knockout (Appl2 −/−) mice, which were viable and grew normally until adulthood, APPL1/APPL2 double knockout mice had normal embryonic development and reproduction [12]. Small interfering RNA mediated knock-down of APPL2 expression in APPL1-null (Appl1(−/−)) murine embryonic fibroblasts (MEFs) consistently resulted in unaltered cell survival rates under normal culture conditions [15]. These results in mice and other vertebrates indicate that the role of APPLs in development maybe species dependent.

Current evidence suggests that APPL1 and APPL2 are not redundant proteins, though they function similarly and act synergistically in the regulation of cellular proliferation, development and apoptosis. In many cases, they play different or even opposite roles to each other. For example, as a binding partner of APPL1 in the BAR domain [3,21], APPL2 exerts opposite effects with APPL1 for insulin- and adiponectin-

stimulated glucose uptake [42] and adiponectin-induced fatty acid oxidation in muscle cells [8]. A detailed overview of the roles of APPL1 and APPL2 in insulin and adiponectin signaling will be provided in the following sections.

3.3. APPLs in immune response

APPL2 has a distinct location and unique functions in macrophages. Both APPLs are involved in regulating the multiple signaling and cytokine outputs from activated macrophages in response to bacterial challenge. Rab 31 is a member of the Rab GTPase family known to regulate post-Golgi trafficking. In addition to its ability to recruit APPL2 to early-stage phagosomes, Rab 31 also interacts with APPL2 to activate PI3K/AKT and to enhance FcγR-mediated phagocytosis of macrophages [43]. APPL2 or Rab 31 depletion reduced PI3K/AKT signaling and phagocytosis, indicating that Rab 31/APPL2 is required for PI3K/AKT activation [43]. Interestingly, the Rab 31/APPL2 complex plays an opposite role from the Rab 5/APPL1 complex as the deletion of APPL1 increased AKT and p38 signaling during phagocytosis [44]. In addition, APPL2 is not only differentially localized from APPL1 but also plays dominant roles in modulating inflammatory responses. In response to LPS treatment, while APPL1 was found mostly on punctuate endosomes and was not recruited to plasma membrane ruffles, APPL2 was recruited to the plasma membrane for the enhancement of plasma membrane ruffles [45]. Specifically, APPL2 was a major effector on the regulation of TLR4 signaling and was a key player in the nuclear translocation of NF-κB p65 and the secretion of pro- and anti-inflammatory cytokines [45].

In contrast, APPL1 plays a major role in the transcription factor interferon regulatory factor 3 (IRF3)-mediated immune response. Innate immune response activated TLR3 and TLR4 trigger the production of pro-inflammatory cytokines through NF-κB, mitogen-activated protein kinase (MAPK), and IRF3signaling pathways in which an aberration in IRF3-dependent gene expression results in a variety of immune diseases [46]. APPL1 was required for both TRIF- and retinoic acid-inducible gene 1-dependent signaling cascades to induce IRF3 expression and activation [47]. In addition, APPL1 was indispensable for IRF3-dependent gene expression in response to some viral and bacterial infections in macrophages in which APPL1 triggered IRF3-dependent gene transcription upon TLR3/4 engagement by recruiting TBK1 and IKKε to endosomes [47]. Furthermore, the transcriptional induction of some IRF3-targeted genes in macrophages infected with the H1N1 virus was also APPL1-dependent [47].

3.4. APPLs in cancer cell invasion

APPL1 and APPL2 possess unique functions in different cancer cells. APPL1 levels were high in aggressive prostate cancer tissues [48]. In prostate cancer cells, APPL1 was required for TGFβ-induced nuclear translocation of the TGFβ type I receptor (TβRI) that contributes to cancer cell invasiveness [48]. High protein and phosphorylation levels of APPL1 were found in tissues from human hepatocellular carcinoma and triple-positive breast cancer [49]. Mechanically, APPL1 directly bound to both the leptin receptor and STAT3, a process that positively mediates leptin signaling and promotes the leptin-induced proliferation and migration of cancer cells [49]. Conversely, expression of APPL2 was up-regulated in approximately 40% of cases of glioblastoma multiforme, a common cancer of the central nervous system [50]. Knockdown of APPL2 in glioma cells decreased cell viability and induced the activation of caspases, which subsequently resulted in apoptotic cell death, suggesting that APPL2 may enhance tumor cell growth and apoptosis resistance [50]. Thus far, the exact roles and mechanisms of APPLs in various cancer cells remain largely unclear, thereby requiring further investigation in the future.

4. APPLs in adiponectin signaling pathways

As one of the most abundant adipokines secreted from adipocytes, adiponectin has numerous protective effects on metabolism. It is well documented that adiponectin possesses *anti*-obesity, anti-inflammatory, anti-proliferation and insulin-sensitizing effects [51,52], which are mainly mediated by its seven transmembrane receptors, AdipoR1 and AdipoR2 [53], and by activating AMP-activated protein kinase (AMPK), MAPK, or peroxisome proliferator-activated receptor-α (PPARα) to stimulate or suppress the downstream signaling pathway cascades [54]. Although both are important for adiponectin action, AdipoR1 and AdipoR2 express and function differently. AdipoR1 is ubiquitously and highly expressed in the skeletal muscle, whereas AdipoR2 is expressed predominantly in the liver [53]. AdipoR1 is involved in activation of AMPK in skeletal muscle, while AdipoR2 is mainly concerned with the regulation and activation of PPARα in the liver, although both of them promote insulin sensitivity [55].

APPL1 was the first adaptor protein identified that interacts directly with adiponectin receptors [18]. A two-hybrid study by Dong and colleagues revealed that the C-terminal extracellular domain of AdipoR1 interacts with adiponectin, whereas the N-terminal cytoplasmic domain of AdipoR1 interacts with APPL1 when stimulated by adiponectin [4]. Importantly, APPL1 interacts with AdipoR1 only when its PTB domain is intact, suggesting that the PTB domain is critical for APPL1 to interact with AdipoR1 [4]. Similarly, APPL1 also interacted with AdipoR2 in a yeast two-hybrid library screen [4], but the interaction domain remains unclear.

The essential roles of APPL1 in adiponectin signaling have been demonstrated in many cells, such as skeletal muscle cells, cardiomyocytes, foam cells, umbilical vein endothelial cells, etc. In muscle cells, the glucose-lowering effects of adiponectin are mainly mediated through promoting glucose uptake by the activation of AMPK [53] and the membrane translocation of glucose transporter 4 (GLUT4) in skeletal muscle [56]. APPL1 enhances the cytosolic localization of LKB1 via its BAR domain thereby facilitating phosphorylation of AMPK [57]. Suppression of APPL1 significantly attenuates the adiponectin-stimulated phosphorylation of AMPK, MAPK, and acetyl-CoA carboxylase (ACC) and fatty acid oxidation [4].

Similarly, adiponectin-stimulated AMPK phosphorylation in the liver is significantly reduced in APPL1 knock-out mice [29]. In hepatocytes treated with adiponectin, APPL1 protein levels are negatively associated with the protein and mRNA levels of gluconeogenesis enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [58]. By modulating the adiponectin-reduced interaction between sirtuin 1 (SirT1) and signal transducer and activator of transcription 3 (STAT3), APPL1 positively regulates adiponectin-stimulated STAT3 activity, thus contributing to the inhibitory effect of adiponectin on hepatic gluconeogenesis [58].

In cardiomyocytes, APPL1 mediates adiponectin induced fatty acid uptake and oxidation [59]. In response to adiponectin, APPL1 binds to AdipoR1 and promotes its interaction with AMPKα2, leading to the phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) and an increase in fatty acid oxidation [59]. Mice fed a high fat diet (HFD) for 16 weeks showed significantly increased myocardial contents of distinct ceramide, sphingomyelin, and diacylglycerol (DAG) species and myocyte dysfunction [60]. Over-expression of APPL1 protected mice from HFD-induced increases in circulating nonesterified fatty acid levels, myocardial lipid accumulation, and cardiac dysfunction [60], although the underlying mechanism of these effects is still unknown. Moreover, APPL1 mediates adiponectin-induced Rho/ROCK-dependent cytoskeleton remodeling to increase glucose uptake and metabolism in cardiomyocytes [61].

In human foam cells, both AdipoR1 and AdipoR2 are involved in the action of adiponectin in reducing lipid accumulation and inhibiting foam cell formation [62]. APPL1 is necessary for adiponectin-suppressed foam cell formation since APPL1 knockdown significantly impairs the

action of adiponectin on lipid accumulation, AKT phosphorylation, and gene expression of scavenger receptor A type 1 (SR-AI) and NF-κB in macrophage foam cells [62]. Thus, the adiponectin-AdipoR1/2-APPL1 axis may be a potential therapeutic target for preventing macrophage foam cell formation and atherosclerosis.

In human umbilical vein endothelial cells, APPL1 serves as a signaling protein that mediates downstream signaling events from adiponectin receptors to eNOS for NO production [23]. RNAi-mediated suppression of APPL1 in endothelial cells blocks adiponectin-stimulated association of heat shock protein 90 (HSP90) with eNOS, which is indispensable for maximal eNOS activity [23]. In cardiac microvascular endothelial cells, APPL1 is involved in adiponectin-regulated cell apoptosis. Adiponectin exerts anti-inflammatory and anti-apoptotic effects via AMPK activation, while interleukin (IL)-18 plays pro-inflammatory and pro-apoptotic roles [63]. Knockdown of APPL1 significantly attenuates adiponectin-induced AMPK phosphorylation and reverses the protective effects of adiponectin on IL-18-induced endothelial cell death, indicating the APPL1-dependent inhibitory effects of adiponectin on vascular injury and inflammation [63].

A number of studies suggest that adiponectin promotes osteoblast differentiation and bone formation by directly targeting GSK-3β and β-Catenin signaling [64,65], favoring bone marrow mesenchymal stem cell (BMSC) differentiation toward the osteoblastic lineage [66,67], decreasing sympathetic tone [67,68], or inducing the production of bone morphogenetic protein 2 (BMP2) in osteoblasts [69]. APPL1 is also involved in adiponectin-induced osteogenic differentiation. In primary cultured h-JBMMSC (human jaw bone marrow mesenchymal stem cells), globular adiponectin promotes osteogenic differentiation by inducing greater expression of osteoblast- related genes such as collagen type I, osteopontin (OPN), bone sialoprotein (BSP), and osteocalcin (OCN), the effects of which are blocked by deletion of APPL1 [70]. Moreover, in the presence of APPL1/tryptophan hydroxylase 2 (TPH2), adiponectin decreases sympathetic tone, increases trabecular bone mass, and promotes osteoblastic commitment of BMSC [67]. Overall, the above findings demonstrate that APPL1 acts as an essential positive regulator of adiponectin-induced osteogenic formation.

Although APPL2 interacts with both AdipoR1 and AdipoR2, it acts as a negative regulator of adiponectin signaling by antagonizing the action of APPL1 [8]. Over-expression of APPL2 inhibits the interaction between APPL1 and AdipoR1, leading to the down-regulation of adiponectin signaling. In addition, suppression of APPL2 expression by RNAi significantly enhances adiponectin-stimulated glucose uptake and fatty acid oxidation [8]. Thus, it is possible that the APPL isoforms act as an integrated Yin-Yang regulator of adiponectin signaling in muscle cells [8]. However, it remains unclear whether this mode of action by APPLs exists in other cells.

5. APPLs in insulin signaling pathways

It is well established that adiponectin is a potent insulin-sensitizing factor. As an essential mediator of adiponectin signaling, APPL1 also plays an important role in insulin signaling pathways. APPL1 enhances insulin sensitivity by promoting the interaction between IR and insulin receptor substrate proteins 1 and 2 (IRS1/2) [29]. APPL1 forms a complex with IRS1/2 under basal conditions that can be recruited to the IR in response to insulin or adiponectin stimulation. The interaction between APPL1 and IR depends on insulin- or adiponectin-stimulated APPL1 phosphorylation, which is greatly impaired in the insulin target tissues in obesity [29]. Deletion of APPL1 in mice leads to systemic insulin resistance and a substantial decrease in insulin-stimulated IRS1/2 tyrosine phosphorylation, indicating that APPL1 sensitizes insulin signaling by acting at a downstream site of the IR [29].

AKT is also a downstream site of the IR and is a key factor in the insulin signaling pathway. Insulin-stimulated AKT kinase activity is dependent on its phosphorylation at Thr308 by PDK1 (phosphoinositide-dependent kinase 1) and at Ser473 by mTOR (mammalian target of rapamycin) [24]. APPL1 improves insulin sensitivity by activating AKT phosphorylation. In C2C12 myocytes, overexpression of APPL1 enhances insulin-stimulated AKT phosphorylation, whereas knockdown of APPL1 or overexpression of APPL1 with a PTB domain mutation significantly reduces the AKT phosphorylation and action of insulin [4]. Consistently, in 3 T3-L1 adipocytes, suppression of APPL1 attenuates insulin-stimulated AKT phosphorylation, GLUT4 translocation, and glucose uptake [24]. In hepatocytes, the insulin-induced activation of AKT and the suppression of gluconeogenesis are enhanced by overexpression of APPL1 in comparison to the attenuation caused by APPL1 knockdown [71]. Specifically, APPL1 interacts with AKT and blocks the association of AKT with its endogenous inhibitor tribble 3 (TRB3) through direct competition, thereby promoting AKT translocation to the plasma membrane and endosomes for subsequent activation [71]. In addition, hepatic overexpression of APPL1 leads to a reduction in hyperglycemia and insulin resistance in obese mice as a consequence of the impaired interaction between AKT and TRB3 [71], suggesting that counteracting the inhibitory action of TRB3 on AKT is an important mechanism by which APPL1 enhances insulin-stimulated suppression of hepatic glucose production. Consistently, chronic exercise increases hepatic APPL1 expression and the interaction between APPL1 and AKT, reducing both TRB3 expression and the association of TRB3 and AKT, thereby contributing to the improvement of insulin sensitivity in the liver [72].

APPL1 is highly expressed in pancreatic β cells, but its levels are significantly decreased in several mouse models of obesity and diabetes, including HFD-induced obese mice and db/db mice [6,73], suggesting that the dysregulation of APPL1 may be associated with malfunction of the pancreas in obesity. Under normal physiological conditions, APPL1 expression in pancreatic beta cells is positively correlated with glucose-induced insulin secretion [6,73]. It is vital in maintaining mitochondrial function in the beta cells as knockdown of APPL1 in INS-1(832/13) cells leads to the significantly down-regulated expression of several genes involved in mitochondrial biogenesis, such as mitochondrial transcription factor A (Tfam) and peroxisome proliferator-activated receptor-γ coactivator-1α (Pgc-1α) [6]. Consequently, the oxygen consumption rate (OCR), maximal mitochondrial respiration capacity, ATP production, and mitochondrial membrane potential (MMP) are all significantly decreased in the APPL1 knockdown cells [6]. In addition, deletion of the APPL1 gene leads to impairment of both the first and second phases of insulin secretion in hyperglycemic clamp tests [6]. In line with these findings, glucose-stimulated insulin secretion (GSIS) and glucose intolerance are significantly decreased in APPL1 knockout mice. Conversely, overexpression of APPL1 prevents HFD-induced glucose intolerance and enhances GSIS [73]. The effects of APPL1 on insulin secretion are associated with its actions on the expression of the exocytotic machinery SNARE proteins (including syntaxin-1, synaptosomal-associated protein 25, and vesicle-associated membrane protein 2) and related exocytosis and insulin-stimulated AKT activation [73]. These data demonstrate that APPL1 may couple insulin-stimulated AKT activation to GSIS by promoting the expression of the core exocytotic machinery during exocytosis. Therefore, it is reasonable to speculate that the obesity-associated reduction of APPL1 expression in pancreatic islets may serve as a pathological link coupling insulin resistance to β-cell dysfunction in obesity and T2D. Nevertheless, the exact mechanism to explain obesity-induced APPL1 reduction remains unclear.

As a critical player in both insulin and adiponectin signaling, APPL1 serves as an important mediator in the cross-talk between these two signaling pathways. On the one hand, APPL1 directly interacts with adiponectin receptors and acts as a positive regulator of adiponectin signaling through the activation of AMPK and p38 MAPK [4,59], leading to increased insulin sensitivity. On the other hand, APPL1 potentiates insulin sensitivity by enhancing insulin-stimulated AKT phosphorylation [4, 24,71] and promoting IRS1/2-IR interaction [29]. Interestingly, treatment of C2C12 cells with adiponectin alone may have no effect on

AKT phosphorylation, while a notable synergistic effect on AKT activation is observed when the cells are treated with both adiponectin and insulin [4]. Furthermore, down-regulation of APPL1 expression by siRNA reduces the synergistic effect of adiponectin on insulin-stimulated AKT phosphorylation [4]. Hence, APPL1-mediated cross-talk between insulin and adiponectin-signaling pathways could be a critical mechanism for the insulin-sensitizing effect of adiponectin.

Regarding insulin-stimulated glucose uptake in muscle cells, APPL2 plays an opposite role compared to APPL1 [42]. Over-expression of APPL2 impairs, whereas deletion of it enhances, the insulin-induced plasma membrane recruitment of GLUT4 and glucose uptake. This process requires the Rab-GTPase-activating protein Tre-2/Bub2/Cdc16 domain family, member 1 (TBC1D1), an interacting partner and downstream effector of APPL2 [42]. The APPL2–TBC1D1 interaction may prevent APPL1/AKT-mediated phosphorylation of TBC1D1 at Thr596, thereby impairing insulin-evoked GLUT4 translation to the plasma membrane [42].

6. Role of APPL in endosomal signaling pathways

Endosomes consist of distinct membrane subdomains within individual organelles and assist in the translation of extracellular stimuli via cytoplasmic transduction cascades to the nucleus [3,74]. Endosomal abnormalities are associated with the pathogenesis of neurological diseases such as Alzheimer's disease and Down Syndrome [75].

The small GTPase Rab 5 is a key factor of membrane transportation from the plasma membrane to the early endosomes, and thus it acts as an essential regulator of cell endolysis and endosomal trafficking [3]. APPL1 and APPL2 are both localized at the cytoplasmic and early endosomal membranes and have been identified to play critical roles in Rab 5-dependent nuclear signal transduction via the endosome [3]. In response to extracellular stimuli (e.g., EGF treatment), APPLs undergo redistribution cycles between endosomes and the nucleus. As a binding partner and effector of Rab 5, APPL1 is released from the membranes by Rab 5-GTP activation and interacts with components of the nucleosome remodeling and histone deacetylase multiprotein complex NuRD-MeCP1, contributing to cell proliferation [3,15]. Therefore, APPL1 functions as a hub linker from the endosome to the nucleus by mediating the Rab 5-trigged signaling pathway.

Rab 5-APPL1 associated endosomal signaling is involved in several neuron-related disorders. Increased amyloid precursor protein (APP) and its cleaved product, β-cleaved carboxy-terminal fragment (βCTF), are associated with the pathogenesis of Alzheimer's disease and Down syndrome [7]. Endosomes are highly active APP processing sites correlated with up-regulation of Rab 5 [7]. By selectively binding to the PTB domain of APPL1 via its YENPTY domain, βCTF recruits APPL1 to Rab 5 endosomes, where APPL1 stabilizes active Rab 5-GTP leading to pathologically accelerated endocytosis, endosome swelling, and selectively impaired axonal transport of Rab 5 endosomes. Nevertheless, these effects could be reversed by knockdown of APPL1 [7]. Therefore, as an essential mediator of Rab 5 activation, APPL1 could be a potential therapeutic target for Alzheimer's disease and Down Syndrome.

APPL associated endosomes also participate in growth factor receptor trafficking and signaling. In the Ras-induced macropinocytic pathway, APPL1 defines a compartment that immediately follows fission from the cell surface and subsequently becomes mature in canonical phosphatidylinositol 3-phosphate (PI3P) positive endosomes [76]. Most APPL endosomes are precursors of classical PI3P positive endosomes, which, in turn, cause APPLs to move back to endocytic vesicles and macropinosomes [76]. PI3P production on endosomes functions as a switch leading to the recruitment of PI3P and Rab 5 to other effectors, the process of which correlates with shedding of APPL1. Hence, depletion of PI3P causes a striking reversion of Rab 5 positive endosomes to the APPL stage, resulting in enhanced growth factor signaling [76]. Similar to APPL1, APPL2 is localized at the cytoplasmic and early endosomal membranes; however, the roles of APPL2 in endosomal trafficking and related signaling are largely unknown and require further investigation.

7. APPL in other signaling pathways

7.1. TrkA signaling

As a receptor of nerve growth factor (NGF), tropomyosin receptor kinase A (TrkA) is a critical factor in the growth and survival of neurons by recruiting signaling molecules such as GIPC PDZ domain containing family, member 1 (GIPC1) and APPL1 [19]. NGF binds to TrkA on axonal terminals, leading to the subsequent activation of MAPK and PI3K /Akt [77,78]. As a newly identified TrkA-interacting protein, APPL1 associates with TrkA through direct binding at the APPL1-PTB domain or indirectly via GIPC1 in a PDZ domain-mediated manner [19]. APPL1 tethers GIPC1 to TrkA with NGF stimulation in neuron cells, which is necessary for the downstream activation of MAPK /extracellular signal-regulated kinase (MEK) and Akt, subsequently promoting neurite outgrowth [9,19].

7.2. EGFR signaling

The epidermal growth factor receptor (EGFR)-mediated signaling pathway regulates multiple biological processes, such as cell proliferation, survival, and differentiation. APPL1 acts to couple receptor trafficking to EGFR signaling via an endocytic compartment [3]. In particular, APPL1 regulates EGFR protein levels in response to EGF stimulation [79]. Over-expression of APPL1 enhances EGFR stabilization while APPL1 depletion reduces EGFR protein levels [79], thus APPL1 is required for EGFR signaling by regulation of EGFR stabilities. This action requires the participation of Rab 5 as APPL1 depletion enhances the activity of Rab 5 that is involved in the internalization and trafficking of EGFR; the inhibition of Rab 5 in APPL1-depleted cells restored EGFR levels [79].

7.3. Wnt/β-catenin signaling

Canonical Wnt/β-catenin signaling regulates many aspects of cellular physiology and tissue homeostasis during development. APPL1 and APPL2 are activators of Wnt/β-catenin/TCF-mediated transcription. Over-expression of APPL1 or APPL2 stimulates the activity of the β-catenin/TCF-dependent reporter construct, whereas silencing of APPL1 reduces it [16]. The stimulatory effects of APPLs on β-catenin/TCF-dependent transcription relay their direct interaction with Reptin, a transcriptional repressor binding to β-catenin and HDAC1 [16]. This interaction decreases the activity of a Reptin-containing repressive complex [16]. In addition, APPL1 interacts and synergizes with Dvl2, an activator of the canonical and non-canonical Wnt pathways, to regulate AP-1-dependent transcription in non-canonical Wnt signaling [11].

7.4. FSH/FSHR signaling

Current evidence suggests that APPLs are closely related to the reproductive system. FSH is essential for ovarian folliculogenesis in females and spermatogenesis in males [80]. The FSHR is a member of the G-protein coupled receptor (GPCR) family and comprises a large extracellular domain and a transmembrane domain [80]. APPL1 and APPL2 interact with FSHR by associating with its trans-membrane domain and regulating the FSHR-mediated signal pathway [20,21]. In this mode of action, APPL1 associates with AKT2, whereas APPL2 interacts with APPL1 via the BAR domains. Subsequently, FSHR, APPL1, APPL2, AKT2 and FOXO1a are organized into complex scaffolding networks in the cell to mediate FSH initiated signal transduction [20,21].

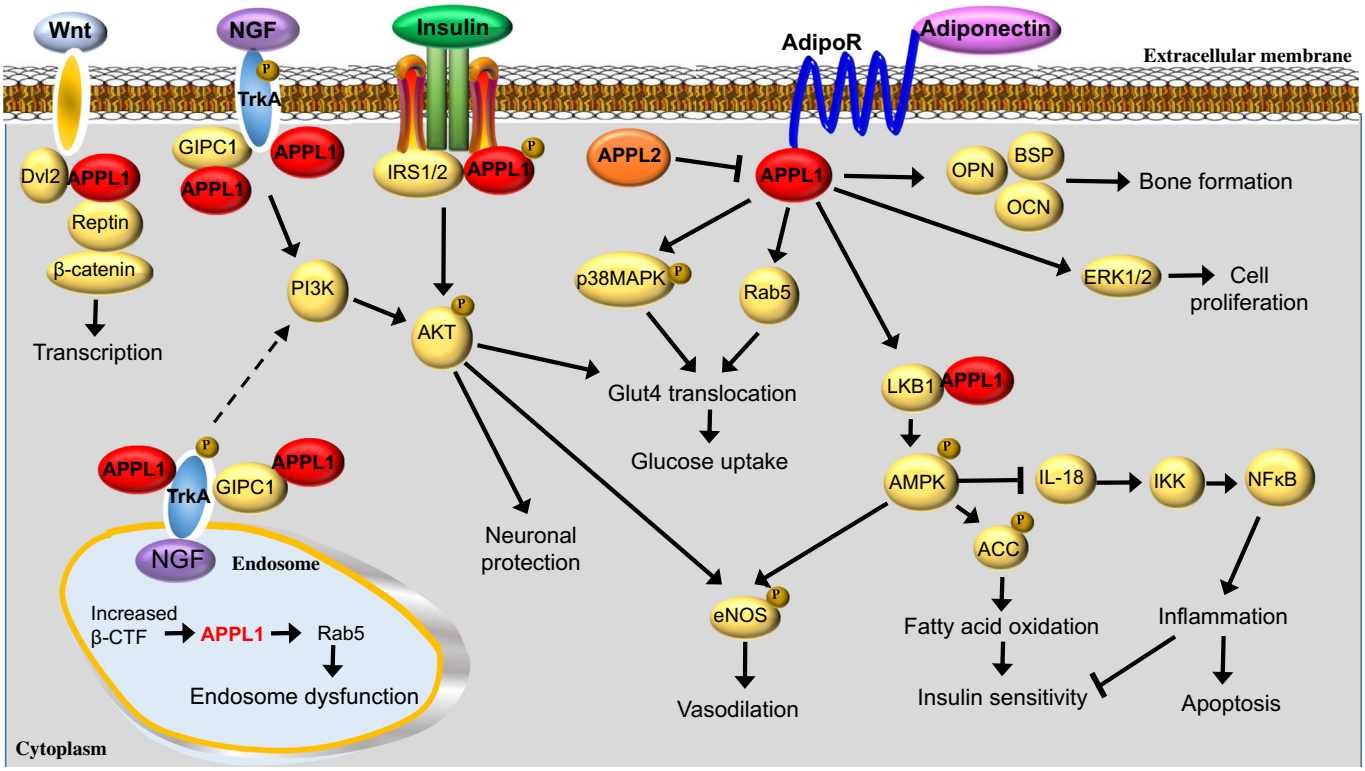

Fig. 2. APPL1-associated signaling cascades. Multifunctional adaptor proteins APPL1 and APPL2 play important roles in many signaling pathways. In response to multiple extracellular signaling, APPL1 directly binds to adiponectin receptors (AdipoR), insulin receptor substrate proteins 1 and 2 (IRS1/2), nerve growth factor (NGF) receptor TrkA, endosomal protein Rab 5, or Wnt and other signaling molecules. These interactions take part in the regulation of glucose/lipid metabolism, vascular functions, cell proliferation and apoptosis, endosomal trafficking, and bone formation. ACC: acetyl-CoA carboxylase; BSP: bone sialoprotein; βCTF, β-secretase-cleaved carboxy-terminal fragment; GIPC1, GAIP-interacting protein; IRS1, insulin receptor substrate proteins 1; OCN, osteocalcin; OPN, osteopontin; and TrkA, tropomyosin receptor kinase A.

Nevertheless, their exact roles in these networks are not fully characterized.

8. Conclusions

Multifunctional adaptor proteins APPL1 and APPL2 play indispensable roles in various signaling pathways in cell proliferation, development, and apoptosis. Notably, APPL1 is not only the adiponectin receptor binding protein but also the essential protein in insulin signaling that mediates the cross talk between the insulin and adiponectin pathways. In addition, APPL1 is involved in endosome trafficking and related signaling transduction, as well as TrkA, Wnt/β-catenin, EGFR and FSHR signaling pathways in many cells and tissues (Fig. 2).

Although recent studies have enriched our understanding of the significant roles played by APPLs in a variety of biological processes, many important aspects of APPL are worth exploring in the future. First, little is known about the regulation of APPL expression and phosphorylation under physiological and pathophysiological conditions. Second, the involvement of APPLs in lipid metabolism is largely unknown, and the investigation of this aspect will clarify the functions of APPLs in adiponectin/insulin regulated lipid metabolism. Third, it is important to characterize the roles of APPL1 and APPL2 in other insulin and adiponectin sensing tissues, such as adipose tissues and the brain. Fourth, the functions of APPLs in the immune response and carcinogenesis are also interesting questions to be addressed.

In the future, animal models with cell/tissue-specific manipulation of APPLs, along with studies related to human samples, will shed light on the roles and specific cellular and molecular mechanisms of APPLs in different cells and tissues. Undoubtedly, more insights into the regulation and function of APPLs will illuminate their associated signaling pathways and develop new strategies for the treatment of obesity, metabolic syndrome, type 2 diabetes, cancer, cardiovascular disease, and neurodegenerative disease.

Conflict of interests

The authors declare that they have no competing interests.

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References

[1] Y. Mitsuuchi, et al., Identification of a chromosome 3p14.3-21.1 gene, APPL, encoding an adaptor molecule that interacts with the oncoprotein-serine/threonine kinase AKT2, Oncogene 18 (35) (1999) 4891–4898.
[2] J. Liu, et al., Mediation of the DCC apoptotic signal by DIP13 alpha, J. Biol. Chem. 277 (29) (2002) 26281–26285.
[3] M. Miaczynska, et al., APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment, Cell 116 (3) (2004) 445–456.
[4] X. Mao, et al., APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function, Nat. Cell Biol. 8 (5) (2006) 516–523.
[5] J. Hennig, et al., APPL proteins modulate DNA repair and radiation survival of pancreatic carcinoma cells by regulating ATM, Cell Death Dis. 5 (2014), e1199.
[6] C. Wang, et al., Deficiency of APPL1 in mice impairs glucose-stimulated insulin secretion through inhibition of pancreatic beta cell mitochondrial function, Diabetologia 56 (9) (2013) 1999–2009.

[7] S. Kim, et al., Evidence that the rab5 effector APPL1 mediates APP-betaCTF-induced dysfunction of endosomes in Down syndrome and Alzheimer's disease, Mol. Psychiatry 21 (5) (2016) 707–716.
[8] C. Wang, et al., Yin-Yang regulation of adiponectin signaling by APPL isoforms in muscle cells, J. Biol. Chem. 284 (46) (2009) 31608–31615.
[9] T. Varsano, et al., GIPC is recruited by APPL to peripheral TrkA endosomes and regulates TrkA trafficking and signaling, Mol. Cell. Biol. 26 (23) (2006) 8942–8952.
[10] A. Schenck, et al., The endosomal protein Appl1 mediates Akt substrate specificity and cell survival in vertebrate development, Cell 133 (3) (2008) 486–497.
[11] M. Banach-Orlowska, E. Szymanska, M. Miaczynska, APPL1 endocytic adaptor as a fine tuner of Dvl2-induced transcription, FEBS Lett. 589 (4) (2015) 532–539.
[12] Y. Tan, et al., Appl1 and Appl2 are expendable for mouse development but are essential for HGF-induced Akt activation and migration in mouse embryonic fibroblasts, J. Cell. Physiol. 231 (5) (2016) 1142–1150.
[13] M. Barbieri, et al., Incretin treatment and atherosclerotic plaque stability: role of adiponectin/APPL1 signaling pathway, J. Diabetes Complicat. (2016).
[14] J. Li, et al., Crystal structures of the BAR-PH and PTB domains of human APPL1, Structure 15 (5) (2007) 525–533.
[15] Y. Tan, et al., Appl1 is dispensable for mouse development, and loss of Appl1 has growth factor-selective effects on Akt signaling in murine embryonic fibroblasts, J. Biol. Chem. 285 (9) (2010) 6377–6389.
[16] S. Rashid, et al., Endosomal adaptor proteins APPL1 and APPL2 are novel activators of beta-catenin/TCF-mediated transcription, J. Biol. Chem. 284 (27) (2009) 18115–18128.
[17] G. Zhu, et al., Structure of the APPL1 BAR-PH domain and characterization of its interaction with Rab5, EMBO J. 26 (14) (2007) 3484–3493.
[18] S.S. Deepa, L.Q. Dong, APPL1: role in adiponectin signaling and beyond, Am. J. Physiol. Endocrinol. Metab. 296 (1) (2009) E22–E36.
[19] D.C. Lin, et al., APPL1 associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction, Mol. Cell. Biol. 26 (23) (2006) 8928–8941.
[20] C.A. Nechamen, et al., Human follicle-stimulating hormone (FSH) receptor interacts with the adaptor protein APPL1 in HEK 293 cells: potential involvement of the PI3K pathway in FSH signaling, Biol. Reprod. 71 (2) (2004) 629–636.
[21] C.A. Nechamen, R.M. Thomas, J.A. Dias, APPL1, APPL2, Akt2 and FOXO1a interact with FSHR in a potential signaling complex, Mol. Cell. Endocrinol. 260-262 (2007) 93–99.
[22] R.B. Jones, et al., A quantitative protein interaction network for the ErbB receptors using protein microarrays, Nature 439 (7073) (2006) 168–174.
[23] K.K. Cheng, et al., Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells, Diabetes 56 (5) (2007) 1387–1394.
[24] T. Saito, et al., The interaction of Akt with APPL1 is required for insulin-stimulated Glut4 translocation, J. Biol. Chem. 282 (44) (2007) 32280–32287.
[25] L. Yang, et al., APPL suppresses androgen receptor transactivation via potentiating Akt activity, J. Biol. Chem. 278 (19) (2003) 16820–16827.
[26] A. Hupalowska, B. Pyrzynska, M. Miaczynska, APPL1 regulates basal NF-kappaB activity by stabilizing NIK, J. Cell Sci. 125 (Pt 17) (2012) 4090–4102.
[27] K.S. Erdmann, et al., A role of the Lowe syndrome protein OCRL in early steps of the endocytic pathway, Dev. Cell 13 (3) (2007) 377–390.
[28] R.L. Gant-Branum, et al., Identification of phosphorylation sites within the signaling adaptor APPL1 by mass spectrometry, J. Proteome Res. 9 (3) (2010) 1541–1548.
[29] J. Ryu, et al., APPL1 potentiates insulin sensitivity by facilitating the binding of IRS1/2 to the insulin receptor, Cell Rep. 7 (4) (2014) 1227–1238.
[30] M. Liu, et al., Phosphorylation of adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif 1 (APPL1) at Ser430 mediates endoplasmic reticulum (ER) stress-induced insulin resistance in hepatocytes, J. Biol. Chem. 287 (31) (2012) 26087–26093.
[31] J. Wang, et al., Serine 707 of APPL1 is critical for the synaptic NMDA receptor-mediated Akt phosphorylation signaling pathway, Neurosci. Bull. 32 (4) (2016) 323–330.
[32] Y.B. Wang, et al., Adaptor protein APPL1 couples synaptic NMDA receptor with neuronal prosurvival phosphatidylinositol 3-kinase/Akt pathway, J. Neurosci. 32 (35) (2012) 11919–11929.
[33] K.K. Cheng, et al., Signaling mechanisms underlying the insulin-sensitizing effects of adiponectin, Best Pract. Res. Clin. Endocrinol. Metab. 28 (1) (2014) 3–13.
[34] G.J. King, et al., Membrane curvature protein exhibits interdomain flexibility and binds a small GTPase, J. Biol. Chem. 287 (49) (2012) 40996–41006.
[35] Q.C. Fang, et al., Association of variants in APPL1 gene with body fat and its distribution in Chinese patients with type 2 diabetic mellitus, Zhonghua Yi Xue Za Zhi 88 (6) (2008) 369–373.
[36] S. Jiang, et al., Genetic variations in APPL2 are associated with overweight and obesity in a Chinese population with normal glucose tolerance, BMC Med. Genet. 13 (2012) 22.
[37] X.W. Ma, et al., Genetic variability in adapter proteins with APPL1/2 is associated with the risk of coronary artery disease in type 2 diabetes mellitus in Chinese Han population, Chin. Med. J. 124 (22) (2011) 3618–3621.
[38] M. Barbieri, et al., Association of genetic variation in adaptor protein APPL1/APPL2 loci with non-alcoholic fatty liver disease, PLoS One 8 (8) (2013), e71391.
[39] B. Wang, et al., Association of APPL1 gene polymorphism with non-alcoholic fatty liver disease susceptibility in a Chinese Han population, Clin. Lab. 61 (11) (2015) 1659–1666.
[40] S. Prudente, et al., Loss-of-function mutations in APPL1 in familial diabetes mellitus, Am. J. Hum. Genet. 97 (1) (2015) 177–185.
[41] L. Wen, et al., Appl1 is essential for the survival of Xenopus pancreas, duodenum, and stomach progenitor cells, Dev. Dyn. 239 (8) (2010) 2198–2207.
[42] K.K. Cheng, et al., The adaptor protein APPL2 inhibits insulin-stimulated glucose uptake by interacting with TBC1D1 in skeletal muscle, Diabetes 63 (11) (2014) 3748–3758.
[43] J.C. Yeo, et al., Rab31 and APPL2 enhance FcgammaR-mediated phagocytosis through PI3K/Akt signaling in macrophages, Mol. Biol. Cell 26 (5) (2015) 952–965.
[44] M. Bohdanowicz, et al., Recruitment of OCRL and Inpp5B to phagosomes by Rab5 and APPL1 depletes phosphoinositides and attenuates Akt signaling, Mol. Biol. Cell 23 (1) (2012) 176–187.
[45] J.C. Yeo, et al., Distinct roles for APPL1 and APPL2 in regulating toll-like receptor 4 signaling in macrophages, Traffic 17 (9) (2016) 1014–1026.
[46] K.A. Fitzgerald, et al., IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway, Nat. Immunol. 4 (5) (2003) 491–496.
[47] T.L. Chau, et al., A role for APPL1 in TLR3/4-dependent TBK1 and IKKepsilon activation in macrophages, J. Immunol. 194 (8) (2015) 3970–3983.
[48] J. Song, et al., APPL proteins promote TGFbeta-induced nuclear transport of the TGFbeta type I receptor intracellular domain, Oncotarget 7 (1) (2016) 279–292.
[49] Y. Ding, et al., APPL1-mediating leptin signaling contributes to proliferation and migration of cancer cells, PLoS One 11 (11) (2016), e0166172. .
[50] B. Pyrzynska, et al., Multifunctional protein APPL2 contributes to survival of human glioma cells, Mol. Oncol. 7 (1) (2013) 67–84.
[51] E. Maury, S.M. Brichard, Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome, Mol. Cell. Endocrinol. 314 (1) (2010) 1–16.
[52] T. Kadowaki, T. Yamauchi, Adiponectin and adiponectin receptors, Endocr. Rev. 26 (3) (2005) 439–451.
[53] T. Yamauchi, et al., Cloning of adiponectin receptors that mediate antidiabetic metabolic effects, Nature 423 (6941) (2003) 762–769.
[54] X. Hui, et al., Adiponectin and cardiovascular health: an update, Br. J. Pharmacol. 165 (3) (2012) 574–590.
[55] T. Yamauchi, et al., Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions, Nat. Med. 13 (3) (2007) 332–339.
[56] R.B. Ceddia, et al., Globular adiponectin increases GLUT4 translocation and glucose uptake but reduces glycogen synthesis in rat skeletal muscle cells, Diabetologia 48 (1) (2005) 132–139.
[57] L. Zhou, et al., Adiponectin activates AMP-activated protein kinase in muscle cells via APPL1/LKB1-dependent and phospholipase C/Ca2+/Ca2+/calmodulin-dependent protein kinase kinase-dependent pathways, J. Biol. Chem. 284 (33) (2009) 22426–22435.
[58] Y. Ding, et al., APPL1-mediated activation of STAT3 contributes to inhibitory effect of adiponectin on hepatic gluconeogenesis, Mol. Cell. Endocrinol. 433 (2016) 12–19.
[59] X. Fang, et al., An APPL1-AMPK signaling axis mediates beneficial metabolic effects of adiponectin in the heart, Am. J. Physiol. Endocrinol. Metab. 299 (5) (2010) E721–E729.
[60] M. Park, et al., APPL1 transgenic mice are protected from high-fat diet-induced cardiac dysfunction, Am. J. Physiol. Endocrinol. Metab. 305 (7) (2013) E795–E804.
[61] R. Palanivel, et al., Adiponectin stimulates Rho-mediated actin cytoskeleton remodeling and glucose uptake via APPL1 in primary cardiomyocytes, Metabolism 63 (10) (2014) 1363–1373.
[62] L. Tian, et al., Adiponectin-AdipoR1/2-APPL1 signaling axis suppresses human foam cell formation: differential ability of AdipoR1 and AdipoR2 to regulate inflammatory cytokine responses, Atherosclerosis 221 (1) (2012) 66–75.
[63] B. Chandrasekar, et al., Adiponectin blocks interleukin-18-mediated endothelial cell death via APPL1-dependent AMP-activated protein kinase (AMPK) activation and IKK/NF-kappaB/PTEN suppression, J. Biol. Chem. 283 (36) (2008) 24889–24898.
[64] Y.Y. Lin, et al., Adiponectin receptor 1 regulates bone formation and osteoblast differentiation by GSK-3beta/beta-catenin signaling in mice, Bone 64 (2014) 147–154.
[65] X.H. Luo, et al., Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway, Exp. Cell Res. 309 (1) (2005) 99–109.
[66] Y. Mitsui, et al., Hyperadiponectinemia enhances bone formation in mice, BMC Musculoskelet. Disord. 12 (2011) 18.
[67] Y. Wu, et al., Central adiponectin administration reveals new regulatory mechanisms of bone metabolism in mice, Am. J. Physiol. Endocrinol. Metab. 306 (12) (2014) E1418–E1430.
[68] M.L. Bouxsein, et al., Guidelines for assessment of bone microstructure in rodents using micro-computed tomography, J. Bone Miner. Res. 25 (7) (2010) 1468–1486.
[69] C.Y. Huang, et al., Adiponectin increases BMP-2 expression in osteoblasts via AdipoR receptor signaling pathway, J. Cell. Physiol. 224 (2) (2010) 475–483.
[70] H. Hu, et al., The osteogenesis effect and underlying mechanisms of local delivery of gAPN in extraction sockets of beagle dogs, Int. J. Mol. Sci. 16 (10) (2015) 24946–24964.
[71] K.K. Cheng, et al., APPL1 potentiates insulin-mediated inhibition of hepatic glucose production and alleviates diabetes via Akt activation in mice, Cell Metab. 9 (5) (2009) 417–427.
[72] R. Marinho, et al., Endurance exercise training increases APPL1 expression and improves insulin signaling in the hepatic tissue of diet-induced obese mice, independently of weight loss, J. Cell. Physiol. 227 (7) (2012) 2917–2926.
[73] K.K. Cheng, et al., APPL1 potentiates insulin secretion in pancreatic beta cells by enhancing protein kinase Akt-dependent expression of SNARE proteins in mice, Proc. Natl. Acad. Sci. U. S. A. 109 (23) (2012) 8919–8924.

[74] M. Lakadamyali, M.J. Rust, X. Zhuang, Ligands for clathrin-mediated endocytosis are differentially sorted into distinct populations of early endosomes, Cell 124 (5) (2006) 997–1009.
[75] R.A. Nixon, The role of autophagy in neurodegenerative disease, Nat. Med. 19 (8) (2013) 983–997.
[76] R. Zoncu, et al., A phosphoinositide switch controls the maturation and signaling properties of APPL endosomes, Cell 136 (6) (2009) 1110–1121.
[77] A. Patapoutian, L.F. Reichardt, Trk receptors: mediators of neurotrophin action, Curr. Opin. Neurobiol. 11 (3) (2001) 272–280.
[78] D.R. Kaplan, F.D. Miller, Neurotrophin signal transduction in the nervous system, Curr. Opin. Neurobiol. 10 (3) (2000) 381–391.
[79] J.R. Lee, et al., Adaptor protein containing PH domain, PTB domain and leucine zipper (APPL1) regulates the protein level of EGFR by modulating its trafficking, Biochem. Biophys. Res. Commun. 415 (1) (2011) 206–211.
[80] J.A. Dias, et al., Emerging roles for the FSH receptor adapter protein APPL1 and overlap of a putative 14-3-3tau interaction domain with a canonical G-protein interaction site, Mol. Cell. Endocrinol. 329 (1–2) (2010) 17–25.