The Adiponectin Signaling Pathway as a Novel Pharmacological Target

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Abstract: There appear to be compelling evidences presenting adiponectin as a key regulator of energy homeostasis. Over the past 10 years, much work has been done to identify the molecular mechanisms by which adiponectin functions in the body. We and other groups have demonstrated that adiponectin activates multiple signaling pathways, which mediate its anti-diabetic, anti-atherogenic and anti-inflammatory functions. Comprehensive analysis of the mechanism of adiponectin action may allow us to elucidate the etiology of metabolic syndrome associated diseases including diabetes and cardiovascular diseases, where dysfunction of adiponectin may contribute to pathogenesis of diseases. While regulation of adiponectin gene expression or secretion remains an interesting topic in studies of cell metabolism, further intensive studies are necessary to illustrate the molecular mechanisms. Importantly, identification of molecules in the adiponectin signaling pathways and in the regulation of adiponectin gene expression may provide novel targets for therapeutic drugs.

Key Words: Adiponectin, signal transduction, insulin, insulin sensitivity, obesity, diabetes.

INTRODUCTION

During recent years, a series of biologically active factors have been identified from adipose tissue, leading to a new definition of adipose tissue as an endocrine organ [1-3]. Adiponectin is one of hormones secreted from the adipose tissue. It is also referred to as adipocyte complement-related protein of 30 kDa (Acrp30) [4], AdipoQ [5], apM1 [6], and gelatin-binding protein-28 (GBP-28) [7]. This hormone is a collagen-like protein that belongs to a superfamily of proteins including a number of members such as C1q A, B, C chains, type VII and type X collagens, and chipmunk hibernation proteins. Adiponectin contains four structural domains based on its primary sequence: an N-terminal signal peptide, a short hypervariable region, a collagen domain, and a C-terminal globular domain homologous to C1q. In mice, knockout of the adiponectin gene leads to insulin resistance [8, 9]. Globular adiponectin transgenic mice showed enhanced insulin sensitivity [10, 11]. In human, the adiponectin gene has been mapped to chromosome 3q27, which is a diabetes-susceptibility locus [12]. Alteration of serum adiponectin levels has been correlated with a number of human diseases, including obesity, diabetes, metabolic syndrome, cardiovascular diseases and cancers (Table **1**). With recent accumulation in understanding the functions of adiponectin, much attention has been attracted to its pharmacological and therapeutic implications. For the regulations and functions of adiponectin, a number of excellent reviews have been published recently [13-18]. The present review focuses on the current understanding of adiponectin signal transduction, as various signaling pathways stimulated by adiponectin have been identified (Fig. (**1**)). Development of drugs targeting the adiponectin pathways may provide novel therapeutic strategies against diseases including diabetes and cardiovascular diseases.

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ADIPONECTIN SIGNALING PATHWAYS

1) The Adiponectin Receptors

As a secreted peptide hormone in plasma, adiponectin functions by binding to its receptors. Kadowaki's group cloned and identified two adiponectin receptors, AdipoR1 and AdipoR2, by screening a skeletal muscle cDNA expression library using globular adiponectin (gAd) [80]. Independently, AdipoR1 was identified in our lab by the yeast two-hybrid approach using full-length adiponectin (fAd) as bait [81]. These two receptors have been predicted to have seven transmembrane domains, with an intracellular N-terminus and an extracellular C-terminus. Due to their low homology to G-protein-coupled receptors (GPCR) families and unique topology on the cell surface, AdipoR1 and AdipoR2 are likely to belong to a new family of receptors.

Despite the fact that AdipoR1 and AdipoR2 appear to be essential for adiponectin functions, the mechanism of how adiponectin signals are transmitted *via* these receptors into cells remains to be elucidated. Unlike protein kinase receptors, AdipoR1 has no detectable tyrosine phosphorylation upon adiponectin stimulation [81]. One of the potential mechanisms could be that binding of adiponectin triggers conformation change of the receptors, which in turn recruits certain adaptor proteins to the receptors and subsequently activates downstream signaling molecules.

In addition to AdipoR1 and AdipoR2, T-cadherin has been reported as a receptor for hexamer and high-molecular-weight forms of adiponectin [82], although the function of this receptor is not well understood. Since T-cadherin is an extracellular protein, it is possible that it serves as a co-receptor of adiponectin receptors, modulating certain adiponectin signals [82]. The existence of other types of adiopnectin receptors remains to be elucidated.

2) APPL1 in the Adiponectin Signaling

Most recently, we have identified an adaptor protein that directly interacts with the intracellular domain of AdipoR1

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Table 1. Diseases Associated with Altered Serum Adiponectin Levels

Decreased Adiponectin levels	Ref.
Type 2 diabetes	19-22
Atherosclerosis	24-27
Essential hypertension	30-32
Breast Cancers	37-39
Obesity	5; 41-43
Nonalcoholic fatty liver disease	49-52
Metabolic syndrome	55-59
Prostate cancer	60; 61
Colorectal adenoma	62
Ischemic heart disease	63
Hypercholesterolemia	64
Gastric cancer	65
Polycystic ovary syndrome	66-69
Gestational diabetes mellitus	70-73
Endometrial cancer	74-76
Obstructive sleep apnea hypopnea syndrome	77-79
Increased Adiponectin levels	**Ref.**
IgA nephropathy	23
Anorexia Nervosa	28; 29
Type 1 diabetes	33-36
Crohn's disease	40
Preeclampsia	44-48
Bulimia nervosa	53; 54

and AdipoR2, *in vitro* and in cells [81]. This protein termed APPL1 (*A*daptor protein containing *P*H domain, *P*TB domain and *L*eucine zipper motif) binds to adiponectin receptors through its PTB domain. Previous studies have shown that APPL1 interacts with Akt and preferentially tethers the inactive form of Akt2 to the p110 subunit of phosphatidylinositol-3 kinase (PI-3 kinase) [83]. In addition, APPL1 may be involved in the PI-3 kinase pathway of follicle-stimulating hormone (FSH) signaling by interaction with FSH receptors [84].

The interaction between AdipoR1 and APPL1 can be stimulated by adiponectin. This interaction is essential for adiponectin-stimulated downstream signaling, such as p38 mitogen-activated protein kinase (p38 MAPK) and 3'-AMP-activated protein kinase (AMPK) activation [81]. APPL1 also mediates adiponectin-stimulated glucose transport 4 (GLUT4) translocation by association with Rab5 [81], a small GTPase that interacts with APPL1 [85]. Our data suggest that APPL1 is critical in adiponectin-stimulated metabolic functions such as glucose utilization and fatty acid oxidation [81].

The APPL1 gene has been mapped to human chromosome 3p14.3-21.1 [83]. Mutations of this region have been correlated with many types of tumors [86]. Expression of APPL1 leads to apoptosis of cancer cells by interaction with DCC (deleted in colorectal cancer) [87], although the molecular mechanism is currently unknown. Interestingly, recent studies have suggested that adiponectin may have antioncogenic properties by activating c-jun N-terminal kinase (JNK), signal transducer and activator of transcription 3 (STAT3), or caspase pathways [88, 89, 90]. Further studies are necessary to investigate whether APPL1 is involved in adiponectin-mediated antitumor activity.

3) AMPK Pathway

Fruebis *et al*. has described for the first time the metabolic effects of adiponectin [91]. Injection of gAd into rodents accelerated free fatty acids oxidation in muscle. Further studies demonstrated that both gAd and fAd stimulate phosphorylation and activation of AMPK on Thr172 in skeletal muscle [92,93]. Activation of AMPK subsequently leads to phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) on Ser79, resulting in an increase in fatty acid oxidation in skeletal muscle. The adiponectin-stimulated phosphorylation and activation of AMPK and ACC are mediated by the interaction of APPL1 with AdipoR1 [81]. Down-regulation of adiponectin-stimulated AMPK and ACC phosphorylation was observed in cultured skeletal muscle of obese and type 2 diabetes, suggesting that adiponectin resistance exists and an impairment of downstream signaling of adiponectin receptors may diminish the effect of adiponectin action [94].

Adiponectin stimulates AMPK activation in a number of tissues. In hepatocytes, only fAd has the potency to regulate phosphorylation and activation of AMPK, thus stimulate free fatty acid oxidation [92, 93]. The AMPK activated by adiponectin stimulation leads to phosphorylation and inactivation of ACCα, which is involved in the regulation of fatty acid synthesis in liver. Blocking AMPK activity with a dominant-negative mutant of AMPK (DN-AMPK) suggests that AMPK also play a critical role in regulating adiponectin-mediated liver gluconeogenesis [95, 96]. In endothelial cells, activation of AMPK is critical for selective inhibition of apoptosis by high molecular weight (HMW) form of adiponectin [97]. Incubation of epididymal rat adipocytes with gAd leads to increased glucose uptake with AMPK-dependent mechanism [92]. In newborn rabbit hearts, gAd was found to stimulate fatty acid oxidation in the absence of insulin, independent of changes in ACC or malonyl-CoA [98]. A study in pancreatic beta cells has shown that activation of AMPK by gAd inhibits cataplerosis of glucose-carbon to lipids [99]. Moreover, adiponectin has been shown to stimulate AMPK in brain and plays a key role in controlling food intake [100,101]. Together, these data indicate that activation of AMPK is a common mechanism for adiponectin mediated diverse functions in various tissues.

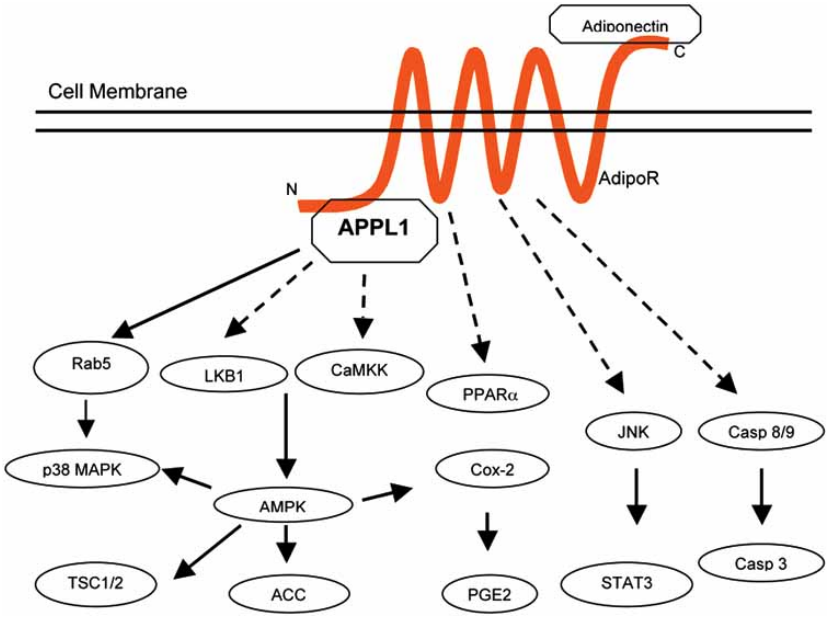

Fig. (1). The adiponection signaling pathways.

The mechanism of adiponectin signals to AMPK remains unclear. It is well known that LKB1 is a major upstream kinase that phosphorylates and activates AMPK on Thr172, a residue in the activation loop [102]. Recently, several studies have established that Ca2+/calmodulin-dependent protein kinase kinase (CaMKK) also phosphorylates and activates AMPK [103-105]. Whether LKB1 or CaMKK is involved in adiponectin-stimulated AMPK activation is currently unknown.

4) MAPK Pathways

Adiponectin has been shown to activate p38 MAPK in C2C12 myotubes [80]. Blocking p38 MAPK activity by a specific inhibitor partially inhibited adiponectin-stimulated glucose uptake and fatty acid oxidation, suggesting p38 MAPK is involved in regulating adiponectin metabolic functions. A recent study has shown that adiponectin stimulates p38 MAPK and JNK that mediate proliferation and differentiation of human osteoblasts [106].

p38 MAPK activation may be mediated *via* direct binding with an adaptor protein [107] or through the classical MAPK pathway [108]. Evidence of how adiponectin stimulates p38 MAPK activation is limited. However, AICAR (Fig. (**2**)), an AMPK activator, has been shown to stimulate p38 MAPK in skeletal muscle [109], suggesting that AMPK could be an upstream activator of p38 MAPK. In cultured cells, treatment of AICAR or overexpression of constitutively active mutant of AMPK (CA-AMPK) resulted in a significant activation of p38 MAPK [110], further supporting the idea that AMPK is located upstream to p38 MAPK in the adiponectin signaling cascades.

We have shown that APPL1 is a critical signaling molecule for p38 MAPK activation stimulated by adiponectin [81]. Overexpression of dominant-negative Rab5 significantly inhibits adiponectin stimulated p38 MAPK activation, indicating that adiponectin activates p38 MAPK partially through regulating the association of APPL1 and Rab5 [81].

Adiponectin may stimulate other members of MAPK family. Previous studies have reported that JNK [88, 105], and STAT3 [88] act as common downstream effectors of adiponectin in prostate cancer cells, hepatocellular carcinoma HepG2 cells, and C2C12 myoblasts. Although the role of JNK activation is not known, inhibition of STAT3 by adiponectin may represent a therapeutic strategy for cancers [88]. Indeed, there is significant inverse correlation between the adiponectin level and prostate-specific antigen levels or biopsy Gleason scores in prostate cancer [61]. Similar correlations were also identified in endometrial [74-76] and breast cancers in women [38, 39, 111, 112]. These data suggest that adiponectin may have antioncogenic properties.

Adiponectin also regulates extracellular signal-regulated kinase (ERK) activity *in vitro* and *in vivo*. In adiponectin-deficient mice, pressure overload resulted in concentric cardiac hypertrophy, probably due to the activation of ERK and diminished AMPK signaling in the myocardium. Supplementation of adiponectin in these mice attenuates cardiac hypertrophy [113]. In cultured cardiac myocytes, the same study revealed that adiponectin inhibited ERK activation induced by norepinephrine in the presence of propranolol or angiotensin II, which could be blocked by DN-AMPK [113]. These data suggest that adiponectin may inhibit ERK activation *via* activation of AMPK. Consistent with this finding, Kim *et al*. have shown that activation of AMPK by AICAR in NIH-3T3 cells resulted in drastic inhibitions of Ras, Raf-1, and ERK activation induced by insulin-like growth factor 1 (IGF-1) [114]. Conversely, inhibition of AMPK by an antisense RNA significantly diminished the AICAR effect on IGF-1-induced ERK activation, implying that adiponectin negatively modulates ERK1/2 activation through AMPK activation. Contradictory to these findings, Chen *et al*. have

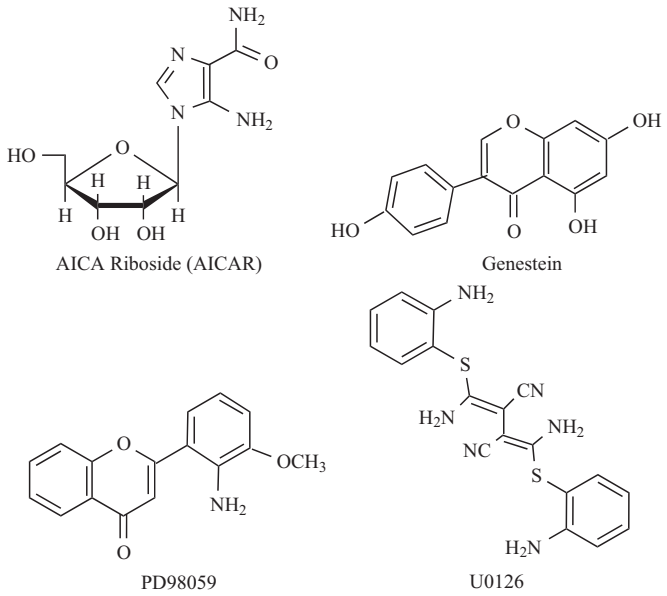

Fig. (2).

reported that AICAR activated ERK and aPKCs in skeletal muscle, which were blocked by tyrosine kinase inhibitor, genistein (Fig. (**2**)), and MEK1 inhibitor, PD98059 (Fig. (**2**)) [115]. In a recent study, AICAR has also been shown to stimulate ERK in oocytes, which could be blocked by MEK inhibitors, PD98059 and U0126 (Fig. (**2**)). These controversial results may be due to the reason that AICAR could specifically modulate signaling pathways other than the AMPK activation pathway in certain type cells.

5) Peroxisome Proliferators-Activated Receptor (PPAR) Pathways

In addition to stimulation of several protein kinases, adiponectin regulates the ligand activity of PPAR. Treatment of C2C12 myocytes with adiponectin markedly increases PPARα ligand activity [80]. In agreement with these observations, administration of adiponectin up-regulated the expression of PPARα target genes, such as CD36, acyl-coenzyme A oxidase, and uncoupling protein 2 [41].

Synthetic PPARγ ligands (TZDs) enhance adiponectin expression in adipocytes [116,117]. In mice lacking adiponectin, the ability of TZDs to improve glucose tolerance is impaired, suggesting that adiponectin mediates the insulin sensitizing effect of TZDs [8]. Interestingly, PPARγ expression is significantly increased in 3T3-L1 fibroblasts overexpressing adiponectin [118]. Furthermore, the rates of proliferation and differentiation in these fibroblasts are enhanced as compared with the control cells. These data indicate that adiponectin may activate the PPARγ pathway through a yet unidentified mechanism, promoting adiponectin expression, and adipocyte differentiation and proliferation in a unique "positive feedback" manner. In type 2 diabetes, decreased adiponectin level may further lead to suppression of adiponectin expression in a so-called vicious cycle [20, 22, 42].

6) NF-κB Signaling

The inhibitory effect of adiponectin on endothelial NF-κB signaling was reported by Ouchi *et al.* [119]. Pretreatment of endothelial cells with adiponectin suppressed TNF-α-induced IκB-α phosphorylation and subsequent NF-κB activation without affecting other TNF-α-mediated phosphorylation signals [119]. Further, the effect of adiponectin on NF-κB is dependent on accumulation of cAMP and subsequent activation of protein kinase A (PKA), indicating that the inhibitory role of adiponectin on NF-κB signaling is through a cAMP-dependent pathway in endothelial cells [119].

Adiponectin attenuates lipopolysaccharide-induced expression and release of TNF-α and interleukin 6 (IL-6) from macrophages, and up-regulates interleukin 10 (IL-10) [120]. These inhibitory effects are mediated by attenuation of NF-κB nuclear translocation and suppression of ERK1/2 activity [120].

The effect of adiponectin on NF-κB regulation in muscle cells is opposite to that in endothelial cells. Treatment of C2C12 myocytes or myotubes with adiponectin led to the activation of NF-κB [121, 122]. Interestingly, only hexamer or HMW form, but not globular or trimer form of adiponectin, are capable of stimulating NF-κB [121, 122].

7) Cyclooxygenase-2 (Cox-2)

Cox-2 is a rate-limiting enzyme in the biosynthesis of prostaglandins, which is significantly induced in response to proinflammatory cytokines or hormones. It catalyzes the conversion of arachidonic acid into PGH_2, which is subsequently converted to various kinds of prostanoids by specific synthases [123]. Yokota and colleagues have observed that recombinant adiponectin blocked fat cell formation in long-term bone marrow cultures and inhibited the differentiation of cloned stromal preadipocytes [124]. This effect is depend-

ent on Cox-2, since inhibition of Cox-2 with a Cox-2 inhibitor prevents this inhibitory action of adiponectin. In addition, adiponectin fails to block fat cell formation from bone marrow cells derived from Cox–2-deficient mice (*COX-2*$^{+/-}$) [124].

In cardiac cells, lipopolysaccharide induces TNF-α expression, which can be inhibited by adiponectin [125]. The inhibitory effect of adiponectin on TNF-α expression is also mediated by Cox-2. Ischemia-reperfusion in adiponectin-deficient mice (ad$^{-/-}$) led to increased myocardial infarct size, myocardial apoptosis and TNF-α expression compared to wild-type mice [125]. Supplement of adiponectin by adenovirus-mediated expression of adiponectin diminishes infarct size, apoptosis and TNF-α production after ischemia-reperfusion in wild type and adiponectin-deficient mice. The cardioprotective effect of adiponectin at least in part depends on Cox-2, which further mediates production of PGE_2. PGE_2 exerts its action *via* specific receptor subtypes encoded by different genes called Eps (EP_1, EP_2, EP_3, and EP_4), all of which belong to the family of GPCRs. The adiponectin effect on TNF-α production is blocked by the EP_4 receptor-selective antagonist, AH23848, suggesting that cardioprotective action of adiponectin is mediated through the adiponectin-Cox-2-PGE_2-EP_4-TNF-α pathway [125].

8) Caspase Signaling

Adiponectin has been shown to have antiatherogenic activity [126]. In patients with cardiovascular diseases, the plasma levels of adiponectin are significantly reduced [127-130]. The vascular-protective activity of adiponectin may result from the protection of endothelial cells from apoptosis, potentially by inhibiting the activity of Caspase 3 [97]. Transduction with DN-AMPK abolished adiponectin-induced reduction of apoptosis in endothelial cells, suggesting that the adiponectin stimulated AMPK pathway mediates the protective role of adiponectin [97].

Interestingly, adiponectin has anti-angiogenic activity and can inhibit endothelial growth *in vitro* or *in vivo* by activation of caspase activity [89]. This study indicated that adiponectin is capable of inducing endothelial apoptosis by initially activating Caspase 8 and subsequently activating Caspase 3 and Caspase 9 in a dose and time dependent manner [89]. The role of adiponectin in regulating caspase activity is still controversial. Its function may be due to different cell systems used in the experiments [97, 89]. Thus, how adiponectin regulates caspase signaling remains to be further investigated.

THE CROSS TALK BETWEEN THE ADIPONECTIN AND INSULIN SIGNALING PATHWAYS

Insulin is a critical hormone that regulates control of energy metabolism and maintenance of normoglycemia and normolipidemia. A clear picture is emerging from the molecular mechanisms that underlie insulin actions at peripheral level from studies of numerous researchers for the past two decades (131). Insulin plays its physiological roles *via* activation of two major signaling pathways in cells: the PI-3 kinase pathway and MAPK pathway (132). Insulin acts by binding to insulin receptor (IR) on the cell surface, resulting in the receptor autophosphorylation that subsequently recruits and/or phosphorylates several substrates in these two pathways.

IRS Proteins (IRS-1, IRS-2, IRS-3 and IRS-4) are the first molecules downstream to IR in the PI-3 kinase pathway. Phosphorylation of IRS proteins on tyrosine residues by insulin receptor provides docking sites for downstream signaling molecule, PI-3 kinase, which sequentially activates several protein serine/threonine kinases in the pathway. Activation of Akt in the PI-3 kinase pathway plays a key role in mediating the physiological functions of insulin, deregulation of which has been shown to eventually cause type 2 diabetes [133].

Adiponectin appears to have significant insulin sensitizing properties. Administration of adiponectin in animal models with type 2 diabetes and insulin resistance significantly enhances insulin effects potentially by enhancing IRS-1 and Akt phosphorylation [134]. Adiponectin deficient mice exhibit insulin resistance due to reduced IRS-1/PI-3 kinase activity in muscle [9]. These data suggest that adiponectin sensitizes insulin signaling by cross talking with the IRS-PI-3 kinase pathway.

Our recent study has shown that APPL1 is a link between adiponectin signaling and the PI-3 kinase pathway [81]. In C2C12 myoblasts, a notable synergistic effect on Akt phosphorylation was observed when the cells were treated with both adiponectin and insulin, and this effect is dependent on APPL1 expression [81]. Interestingly, APPL1 has also been shown to associate with signaling molecules such as Akt and PI-3 kinase [83] in the insulin signaling pathway, and to be possibly involved in the PI-3 kinase pathway of FSH signaling [84]. As APPL1 is an adaptor protein containing multiple functional domains, it is possible that APPL1 may function as a scaffold protein to manipulate the cross talk between insulin pathways and adiponectin pathways.

Insulin may act as a pro-survival factor in the heart. Insulin decreases apoptosis in isolated rat neonatal cells subjected to hypoxia and then re-oxygenation *via* PI-3 kinase dependent Akt phosphorylation [136]. Interestingly, adiponectin also exerts a protective effect against myocardial cell apoptosis, infarction and inflammation during ischemia reperfusion injury. These actions are mediated through AMPK- and Cox-2-dependent mechanisms [137]. It is possible that the adiponectin-mediated AMPK-Cox-2 pathway and insulin-mediated PI-3 kinase-Akt pathway converge to up-regulate pro-survival factors that enhance insulin action on cell apoptosis.

Adiponectin has also been shown to regulate MAPK signaling pathways. In osteoblasts, adiponectin induces two of the mitogen-activated kinases, namely p38 MAPK and JNK, resulting in increased cell proliferation and differentiation [135]. It has been reported that JNK activation results in impairment of GLUT4 translocation and fatty acid induced insulin resistance [138]. In addition, the level of adiponectin secretion is drastically reduced. Inhibition of JNK activation partially rescues adiponectin mRNA expression [139]. JNK-interacting protein (JIP) is a scaffolding protein that interacts with JNK and mediates its activation. It has been implicated in the regulation of adiponectin. JIP (-/-) mice exhibit increased insulin sensitivity and increased Akt phosphoryla-

tion. The levels of plasma adiponectin concentration are also induced as compared to control [140]. Taken together, these studies imply that the adiponectin signaling pathway is involved in the cross talk with the JNK pathway and the regulation of JNK activity is one of the mechanisms controlling serum adiponectin concentration.

A cross talk between AMPK in the adiponectin pathway and Akt in the insulin signaling pathway in response to adiponectin stimulation in umbilical vein endothelium cells has been previously described [141], although an Akt-independent mechanism may also exist [142]. Adiponectin may stimulate angiogenesis [141] and endothelial nitric oxide (eNO) production [142] through this cross talk. Inhibition of AMPK pathway prevented Akt activation, thus linking AMPK as an upstream activator of Akt. Furthermore, activation of Akt appears to be dependent on the PI-3 kinase [141]. Therefore, adiponectin's pro-angiogenic property is mediated through the AMPK-PI3K-Akt-eNOS pathway in endothelial cells.

In addition, activation of AMPK by AICAR treatments has been shown to suppress protein synthesis by down-regulation of mTOR/S6K, downstream signal molecules of PI-3 kinase pathway, in rat skeletal muscle [143]. In agreement with this finding, S6K1-deficient mice displayed super sensitivity to insulin and are protected against diet induced obesity [144], suggesting S6K1 may negatively modulate insulin sensitivity in a way of "negative feedback". Activated AMPK by AICAR or adiponectin may subsequently phosphorylate and activate TSC2 [145], which together with TSC1 serves as a GTPase-activating protein and further inhibits Rheb activity. Overexpression of the small GTPase, Rheb, has been shown to induce S6K activation and inhibits Akt activation induced by insulin [146]. Therefore, inhibition of mTOR/S6K signaling stimulated by adiponectin induced AMPK activation may be responsible for its insulin sensitizing properties (Fig. (**3**)).

REGULATION OF ADIPONECTIN GENE EXPRESSION

Adiponectin was found to circulate in human plasma at high concentrations, ranging from 1.9 to 17.0 μg/ml, which accounts for approximately 0.01% of the total plasma protein [43]. Plasma concentration of adiponectin may depend on its expression in and secretion from adipocytes, as well as its turnover rate in the blood. Regulation of adiponectin expression or secretion has been intensively investigated since the report that adiponectin mRNA expression in white adipose tissue was paradoxically lower in ob/ob mice, as compared to the wild-type mice [5]. However, the molecular mechanism is not completely elucidated.

Expression or secretion of adiponectin might be controlled by a number of factors (Fig. (**4**)). Treatment of cultured human visceral adipose tissue with insulin or IGF1 for 24 hrs enhanced adiponectin mRNA expression [147]. Phosphorylation of C/EBPbeta at a consensus ERK/GSK3 site (T188) has been implicated in regulation of adiponectin expression by insulin [148]. Chronic administration of growth hormone significantly increased adiponectin expression in a dose-dependent manner, which is mediated by Janus kinase2 (JAK2) and the p38 MAPK [149]. In addition, AICAR and TZDs may also promote adiponectin gene expression in adipocytes [150-151]. Interestingly, adiponectin expression or secretion is physiologically up-regulated by exercise and cold exposure, as well as caloric restriction or weight reduction [152-162].

Conversely, expression or secretion of adiponectin is down-regulated by inflammatory cytokines such as TNF-α and IL6 [163]. Regulation of adiponectin expression by TNF-α is probably mediated by JNK, since inhibition of JNK activity abolished the inhibitory effect of TNF-α on adiponectin expression [164]. It has been demonstrated that adiponectin stimulates JNK activation, which might repre-

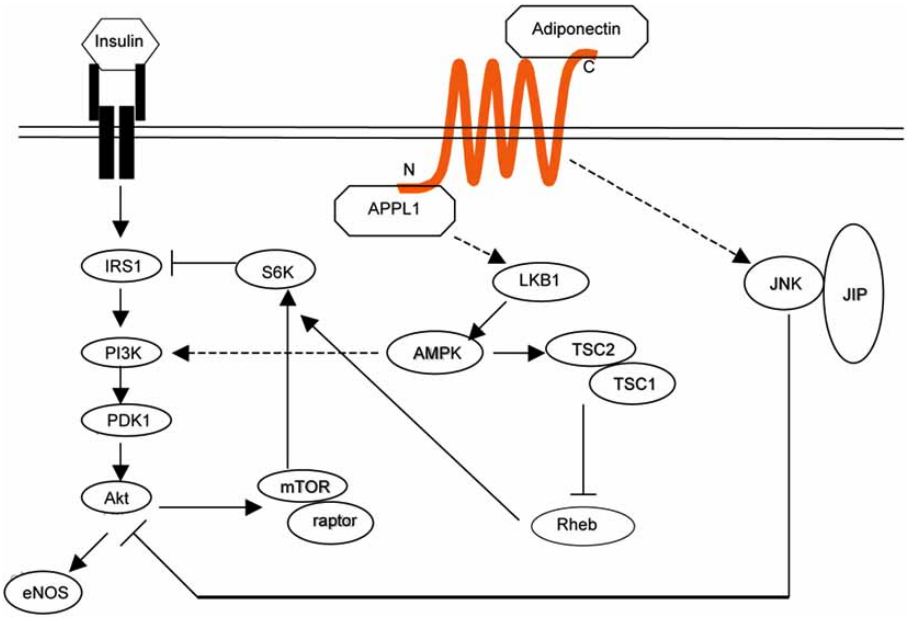

Fig. (3). A cross talk between the adiponectin and insulin signaling pathways.

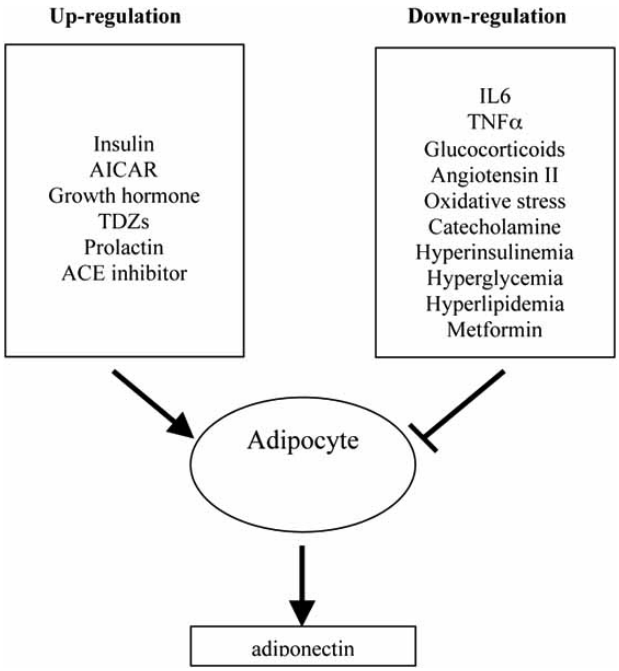

Fig. (4). Regulation of the adiponectin gene expression and secretion.

sent a "negative feedback" mechanism of adiponectin expression. Similarly, a recent study has been able to show that metformin-induced suppression of adiponectin expression and release is mediated by AMPK [165]. In addition, oxidative stress leads to decreased adiponectin expression [166-167], which could be prevented by antioxidants [167]. It has been well established that oxidative stress activates AMPK, p38 MAPK, JNK, and NF-κB, which can also be downstream effectors of adiponectin (Fig. (**1**)). Furthermore, adiponectin expression and secretion are down-regulated by high levels of insulin, glucose, and free fatty acids [168-169], as well as glucocorticoids (GCs) and catecholamines [147, 170-172].

Collectively, adiponectin expression is highly regulated by many factors including insulin, GH, PRL, TNF-α, GCs and catecholamines (Fig. (**4**)). These factors regulate adiponectin expression by regulating the binding of transcriptional factors, such as PPARγ, C/EBPß, and adipocyte determination- and differentiation-dependent factor 1/sterol regulatory element-binding protein 1c (ADD1/SREBP1c) [173], to the critical regulatory sites of adiponectin gene. Interestingly, altered levels of these factors have been implicated in insulin resistant states. Up-regulation of adiponectin expression by therapeutic drugs towards improving insulin sensitivity (such as TDZs) plays a significant role in their insulin sensitizing effects. Recently, it has been shown that mice lacking adiponectin are resistant to TDZs [8]. Weight reduction, caloric restriction and exercise have also been demonstrated to improve insulin sensitivity probably by up-regulation of adiponectin [152-162]. Thus, regulation by targeting adiponectin expression may become a promising therapeutic tool for metabolic syndrome associated diseases in the future, given that adiponectin is a key factor that maintains energy homeostasis.

CONCLUDING REMARKS

Adiponectin stimulates multiple pathways, including the AMPK, PPARα/γ, and p38 MAPK pathway. These pathways may be tissue-specific, which mediate the physiological functions of adiponectin in the body. However, the molecular mechanism has not been fully elucidated.

While adiponectin has been shown to sensitize insulin action, it has also been proposed that a status called "adiponectin resistance" could be an additional setback for treatment of diabetes or insulin resistance using adiponectin. Expression or secretion of adiponectin by adipocytes is highly regulated, representing an alternative therapeutic approach to improve insulin sensitivity. Thus, understanding the molecular mechanism of adiponectin signaling pathways as well as adiponectin gene expression will not only help to understand better the etiology of metabolic syndromes but also may provide new therapeutic strategies for related diseases.

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