Adiponectin signals in prostate cancer cells through Akt to activate the mammalian target of rapamycin pathway

D Barb1, A Neuwirth1, C S Mantzoros1 and S P Balk2

1Division of Endocrinology, Diabetes and Metabolism Biology Program and 2Cancer Biology Program, Hematology-Oncology Division, Department of Medicine, Harvard Medical School, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, Massachusetts 02215, USA

(Correspondence should be addressed to S P Balk; Email: sbalk@bidmc.harvard.edu)

D Barb, A Neuwirth and C S Mantzoros contributed equally to this work

Abstract

Adiponectin has received much attention due to its beneficial effects on insulin sensitivity, and epidemiologic studies have further shown an inverse association between adiponectin levels and risk for multiple tumors, which is independent of the IGF system or other risk factors. Previous studies have shown that adiponectin can activate AMP-activated protein kinase (AMPK) in myocytes, hepatocytes, and adipocytes, suggesting that adiponectin may suppress tumor development through AMPK activation and subsequent inhibition of mammalian target of rapamycin (mTOR). However, the mechanisms through which adiponectin affects cancer cells are not understood, and it remains to be determined whether adiponectin is linked to the same downstream targets in all cells types, and in particular in cancer cells. In the present study, we demonstrate that while adiponectin stimulates AMPK in phosphatase and tensin homolog deleted on chromosome ten (PTEN) deficient LNCaP prostate cancer cells, it also increases mTOR activity as assessed by phosphorylation of two downstream targets, p70 S6 kinase and ribosomal protein S6. This adiponectin stimulation of mTOR was mediated through phosphatidylinositol 3-kinase (PI3 kinase) and Akt activation. These results show that adiponectin can activate both AMPK and PI3 kinase/Akt pathways, and that cell type-specific factors such as PTEN status may determine which of these pathways will have the dominant effect on mTOR. Therefore, while it is possible that high endogenous adiponectin levels could be protective against cancer by direct mechanisms or indirect systemic mechanisms, our results indicate that adiponectin may also directly stimulate signaling pathways that enhance the growth of some tumors.

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Introduction

The adipocyte-secreted protein adiponectin (Acrp30, apM1 protein, adipoQ, or GBP28; Scherer et al. 1995, Hu et al. 1996, Maeda et al. 1996, Nakano et al. 1996) has gained much attention as a marker for insulin resistance and cardiovascular disease (Kadowaki & Yamauchi 2005). Notably, adiponectin is one of the most abundant serum proteins with plasma levels in healthy humans in the microgram per milliliter range (≈ 10 μg/ml; Gavrila et al. 2003a). It exists and circulates in trimers that form dimers (hexamers) and higher order oligomers referred to as high-molecular weight (HMW) adiponectin (Scherer et al. 1995, Kadowaki & Yamauchi 2005). The active form in humans appears to be the full-length HMW form, and potency has been linked to posttranslational modifications such as glycosylation and hydroxylation (Pajvani et al. 2004). Proteolytic cleavage of the full-length molecule yields a smaller globular fragment (globular adiponectin), a form that is generated when the protein is produced in bacteria, but not in mammalian cells under normal conditions (Kadowaki & Yamauchi 2005). Although its role has not been definitively established, accumulating evidence suggests that adiponectin regulates insulin sensitivity. Adiponectin-knockout mice develop glucose intolerance, hyperlipidemia, and increased susceptibility to vascular injury, while adiponectin
The mechanisms underlying adiponectin’s insulin-sensitizing effects or through which adiponectin may affect cell proliferation are not completely understood. It is thought that adiponectin improves insulin sensitivity and increases fatty acid oxidation through phosphorylation and activation of AMP-activated protein kinase (AMPK; Berg et al. 2001, Yamauchi et al. 2002, Wang et al. 2007), p38 MAP kinase activation, and peroxisome proliferator-activated receptor α (PPARα) effects (Yamauchi et al. 2002, 2003, Yoon et al. 2006). Activated AMPK inhibits enzymes that regulate protein, fatty acid, and triglyceride synthesis, including mammalian target of rapamycin (mTOR), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and glycerol phosphate acyltransferase (Luo et al. 2005b). In addition, activated AMPK positively regulates two important proteins for the control of growth arrest and apoptosis, p53 and p21 (Luo et al. 2005b).

Activation of the phosphatidylinositol 3-kinase (PI3 kinase)/Akt/mTOR pathway plays a major role in the development of many cancers, with loss of PTEN being a common mechanism for activation of this pathway in prostate cancer (Vivanco & Sawyers 2002). As lower levels of adiponectin are associated with increased cancer risk (see above), we hypothesized that higher levels of adiponectin may inhibit tumor development or growth through activation of AMPK and subsequent downregulation of mTOR. To test this hypothesis, we examined LNCaP prostate cancer cells that have high levels of basal mTOR activity due to PTEN loss and subsequent activation of the PI3 kinase/Akt/mTOR pathway. In the present study, we demonstrate that adiponectin stimulates AMPK phosphorylation in these cells, but unexpectedly find that adiponectin does not inhibit, but rather activates the mTOR pathway.

Materials and methods
Cell culture and reagents
LNCaP cells, derived from a metastatic prostate cancer, were obtained from the American Type Culture Collection (Manassas, VA, USA) and were cultured in a 5% CO2 incubator in RPMI 1640 with 10% fetal bovine serum (FBS) or 10% steroid hormone-depleted charcoal dextran-stripped FBS (Hyclone, Logan, UT, USA). CWR22Rv1 cells were derived from the CWR22 human prostate cancer xenograft and were grown in Dulbecco’s modified Eagle’s medium (DMEM) with 5% FBS (Sramkoski et al. 1999). Hepatocellular carcinoma (HepG2) cells were from the

treatment improves insulin resistance, lipid, and vascular abnormalities (Kubota et al. 2002, Maeda et al. 2002). Studies in humans and non-human primates have shown a strong negative correlation of adiponectin levels with fasting glucose, insulin, and insulin resistance, independently from body mass index, and development of the metabolic syndrome (Hotta et al. 2001, Cnop et al. 2003, Gavrila et al. 2003b, Luo et al. 2005b).

Circulating adiponectin levels are also decreased in some cancers, in addition to decreases in obesity, type 2 diabetes, and cardiovascular disease (Barb et al. 2006). Epidemiological studies have shown an inverse association between the adiponectin levels and the risk for multiple cancers, including endometrial, breast, prostate, gastric, and renal cancers (Miyoshi et al. 2003, Petridou et al. 2003, 2006, Dal Maso et al. 2004, Mantzoros et al. 2004, Freedland et al. 2005, Goktas et al. 2005, Ishikawa et al. 2005, Cust et al. 2006, Soliman et al. 2006, Korner et al. 2007, Michalakis et al. 2007, Spyridopoulos et al. 2007). One prospective study showed that low adiponectin levels correlated with increased risk for colorectal cancer (Wei et al. 2005), while another study in a different population did not confirm this finding (Lukanova et al. 2006). The two putative adiponectin receptors (AdipoR1 and AdipoR2) are highly expressed not only in metabolically active organs, i.e., skeletal muscle, liver, adipose tissue, pancreas (Yamauchi et al. 2003), and more recently in the brain (Ahima 2006), but also in breast (Dieudonne et al. 2006), prostate (Miyazaki et al. 2005, Bub et al. 2006, Mistry et al. 2006), and hepatocellular carcinomas (Miyazaki et al. 2005). Thus, in addition to its insulin-sensitizing role, adiponectin may also regulate cell proliferation and specific signaling pathways in cancer cells.

Studies in vivo showed that intratumoral administration of adiponectin to mice resulted in suppression of a fibrosarcoma tumor (Brakenhielm et al. 2004) and exogenous adiponectin suppressed the growth of myelomonocytic leukemia cells (Yokota et al. 2000). Two recent studies found that adiponectin suppressed cell growth in the MDA-MB-231 (Kang et al. 2005) and MCF-7 (Dieudonne et al. 2006) breast cancer cell lines; but only the HMW oligomer of adiponectin inhibited proliferation of an androgen-dependent (LNCaP-FGC) as well as two androgen-independent (DU145 and PC-3) prostate cancer cell lines (Bub et al. 2006). However, it should be noted that the latter studies were performed under serum-free conditions, which may favor a decrease in cell proliferation and/or inhibit cell growth. In contrast, both full-length and globular adiponectin stimulated colonic (HT-29) epithelial cell proliferation (Ogunwobi & Beales 2006).

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LNCaP cells, derived from a metastatic prostate cancer, were obtained from the American Type Culture Collection (Manassas, VA, USA) and were cultured in a 5% CO2 incubator in RPMI 1640 with 10% fetal bovine serum (FBS) or 10% steroid hormone-depleted charcoal dextran-stripped FBS (Hyclone, Logan, UT, USA). CWR22Rv1 cells were derived from the CWR22 human prostate cancer xenograft and were grown in Dulbecco’s modified Eagle’s medium (DMEM) with 5% FBS (Sramkoski et al. 1999). Hepatocellular carcinoma (HepG2) cells were from the
American Type Culture Collection and were grown in DMEM with 10% FBS. Recombinant human full-length adiponectin/Acrp30 was purchased from R&D Systems (Minneapolis, MN, USA) and from Biovendor Laboratory Medicine Inc. (Candler, NC, USA) and was diluted in DMEM with 0.1% BSA. 5-aminomidazole-4-carboxamide-1-ß-n-ribonucleoside (AICAR) and antibodies against phospho-AMPK (AMPK ß; Thr172), phospho-ACC (Ser79), S6, phospho-S6 (Ser235/Ser236), phospho-p70 S6 kinase (p70 S6K; Thr421/Ser424), phospho-p70 S6 Kinase (Thr389), p44/42 MAP kinase (MAPK; Erk1 and Erk2), phospho-p44/p42 MAPK (Thr202/Tyr204), Akt, and phospho-Akt (Thr308 and Ser473) were purchased from Cell Signaling Technology (Beverly, MA, USA) and were used at dilutions recommended by the manufacturer. Another phospho-S6 antibody targeting residues Ser 240/244 was also from Cell Signaling, and was used in Fig. 2. Antibodies against ß-tubulin (D-10) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell treatments

Cells were cultured in 24-well plates to ~80% confluence as specified above, and were then serum starved (0.1% FBS) for 24 h prior to treatment. Cells were treated for varying times as indicated with adiponectin at concentrations ranging from 1 to 20 µg/ml, with untreated controls receiving the same volume of DMEM with 0.1% BSA. AICAR was used at 2 mM, rapamycin was used at 10–20 nM, LY294002 at 2 mM, and was diluted in DMEM with 0.1% BSA. 5-aminimidazole-4-carboxamide-1-ß-n-ribonucleoside (AICAR) and antibodies against phospho-AMPK (AMPK ß; Thr172), phospho-ACC (Ser79), S6, phospho-S6 (Ser235/Ser236), phospho-p70 S6 kinase (p70 S6K; Thr421/Ser424), phospho-p70 S6 Kinase (Thr389), p44/42 MAP kinase (MAPK; Erk1 and Erk2), phospho-p44/p42 MAPK (Thr202/Tyr204), Akt, and phospho-Akt (Thr308 and Ser473) were purchased from Cell Signaling Technology (Beverly, MA, USA) and were used at dilutions recommended by the manufacturer. Another phospho-S6 antibody targeting residues Ser 240/244 was also from Cell Signaling, and was used in Fig. 2. Antibodies against ß-tubulin (D-10) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Immunoblotting

Total protein content in each lysate was determined using the bicinchoninic acid assay (Pierce, Rockford, IL, USA) and then lysates containing equal amounts of protein were separated on NuPAGE Novex high-performance precasted gels (Invitrogen). After transfer to nitrocellulose membranes, blots were blocked with 5% nonfat milk in 0.2% Tween 20 in Tris-buffered saline (TBS) for 1 h and then incubated with primary antibody at 4 °C overnight. Blots were then washed six times for 10 min in washing buffer (0.2% Tween 20 in TBS), followed by incubation for 2 h at room temperature with a specific secondary antibody, either anti-mouse or anti-rabbit horseradish peroxidase (Promega). Detection was carried out with enhanced chemiluminescence (ECL) detection reagents (Perkin–Elmer Life Sciences, Boston, MA, USA). In some cases, developed blots were stripped in 62.5 mM Tris–HCl (pH 6.8), 2% SDS, and 0.85% ß-mercaptoethanol at 55 °C for 30 min and reused for immunoblotting to confirm equal protein loading. The changes observed were consistent, and the data shown are representative of at least three independent experiments.

Results

Adiponectin increases AMPK phosphorylation in LNCaP prostate cancer cells

The signaling pathways downstream of the adiponectin receptor are not yet well understood, but previous studies have shown that adiponectin can stimulate the phosphorylation and activation of AMPK in metabolically active cells such as myocytes, hepatocytes (Yamauchi et al. 2002), and adipocytes (Wu et al. 2003). AMPK activation is one of the mechanisms by which adiponectin may exercise its insulin-sensitizing effects by increasing fatty acid oxidation, increasing glucose uptake in skeletal muscle, reducing molecules involved in gluconeogenesis in the liver, and enhancing insulin receptor signaling (Berg et al. 2001, Yamauchi et al. 2002, Wang et al. 2007). AMPK has also been implicated in the pathogenesis of the metabolic syndrome and cancer, but it remains to be determined whether adiponectin affects the same downstream targets in all tissues and cells, and in particular in cancer cells (Luo et al. 2005b, Dieudonne et al. 2006). Therefore, we examined adiponectin stimulation of AMPK in LNCaP prostate cancer cells, which are PTEN deficient and provide a model of advanced prostate cancer.

LNCaP cells were serum starved for 24 h in medium with 0.1% FBS, then treated with adiponectin over a time course from 15 to 60 min, and AMPK activation was measured by immunoblotting with an AMPK ß subunit phospho-specific antibody against phospho-threonine 172. AICAR treatment was used as a positive control. Adiponectin treatment at 1 or 10 µg/ml stimulated an increase in AMPK phosphorylation that was evident at between 15 and 30 min (Fig. 1A). Interestingly, AMPK phosphorylation at the higher concentration of adiponectin appeared to be already decreasing at 30 min, consistent with a transient signal that is rapidly downregulated. As shown in Fig. 1B, the maximal response at 1 µg/ml adiponectin was at between 10 and 30 min.
ACC is one of the major downstream targets of AMPK and is directly phosphorylated by AMPK at serine 79. Therefore, to confirm that AMPK was being activated in response to adiponectin, we also examined ACC phosphorylation. Consistent with AMPK activation, adiponectin stimulated phosphorylation of ACC at serine 79 in the LNCaP cells (Fig. 1C). The magnitude of the stimulation by adiponectin was similar to AICAR at 15 min and was still detectable after 60 min. Further adiponectin stimulations were then done to correlate the phosphorylation of AMPK and ACC, and to confirm that these short-term stimulations were not changing total levels of AMPK or ACC. While there was some variation in the precise magnitude and time course of responses to adiponectin, robust AMPK activation by 1–5 μg adiponectin was consistently observed within 15–30 min (Fig. 1D). The increase in phosphorylation of ACC was less marked than the increase in AMPK, but was observed at the same time. Finally, there were no clear increases in the total levels of AMPK or ACC in response to adiponectin, with total ACC levels decreasing at the higher level of adiponectin.

Adiponectin signaling activates mTOR in LNCaP cells

A consequence of AMPK activation is suppression of cell proliferation, which involves multiple mechanisms including regulation of the cell cycle and inhibition of protein and de novo fatty acid synthesis (Luo et al. 2005b). Cell cycle regulation by AMPK is mediated by upregulation of the p53–p21 axis as well as regulation of the tuberin/tuberous sclerosis complex 2 (TSC2)/mTOR pathway (Xiang et al. 2004). AMPK phosphorylates and activates TSC2, which leads to inhibition of mTOR and its downstream effector kinase (p70 S6 kinase), thus negatively regulating protein synthesis (Luo et al. 2005b). In addition, AMPK may directly phosphorylate and inhibit mTOR (Cheng et al. 2004). AICAR, an activator of AMPK, was shown previously to inhibit mTOR and p70 S6 kinase and to decrease the expression of ACC and FAS by 24–72 h in LNCaP cells (Xiang et al. 2004). Therefore, we next tested the hypothesis that adiponectin can rapidly inhibit the mTOR pathway through activation of AMPK in LNCaP cells.

Surprisingly, we found that although AMPK was activated by short-term treatment with adiponectin, mTOR activity was increased as measured by phosphorylation of its downstream target, p70 S6 kinase (Fig. 2A). Phosphorylation of ribosomal protein S6, the major target of p70 S6 kinase, was also increased by adiponectin. Significantly, mTOR activation was not observed when AMPK was activated by AICAR, with no change or a decrease in most experiments, indicating that mTOR was being activated by adiponectin independently of AMPK. The adiponectin-mediated activation of p70 S6 kinase and subsequent S6 phosphorylation were confirmed with another phos- pho-S6 antibody recognizing Ser 240/244 (Fig. 2B). Finally, this increase in S6 phosphorylation did not reflect an increase in total S6, as these levels were actually moderately decreased in response to adiponectin. Similar effects were observed upon stimulation with IGF-I, a well-established activator of the PI3 kinase/Akt/mTOR pathway (Fig. 2B).

To confirm that the S6 phosphorylation observed in response to adiponectin-reflected mTOR activation,
we used the mTOR inhibitor rapamycin. Importantly, adiponectin did not stimulate S6 phosphorylation in LNCaP cells treated with 10 or 20 nM rapamycin (Fig. 3A). Indeed, S6 phosphorylation was modestly decreased by adiponectin in the rapamycin-treated cells, suggesting a dominant effect of AMPK on suppression of mTOR under these conditions. Significantly, AICAR decreased the basal level of S6 phosphorylation in the same experiment, showing that AMPK can negatively regulate mTOR in these cells (Fig. 3B).

**Adiponectin activates the PI3 kinase/Akt pathway in LNCaP cells**

Another major upstream regulator of mTOR is the PI3 kinase/Akt pathway. Akt is activated by phosphorylation on threonine 308 and serine 473 in response to PI3 kinase activation. Akt can then phosphorylate and suppresses the activity of tuberin. Tuberin (TSC2) and hamartin (TSC1) function together as negative regulators of Rheb, a small GTPase that activates mTOR, so that Akt suppression of tuberin results in mTOR activation (Inoki et al. 2002, Potter et al. 2002). Therefore, we next determined whether the PI3 kinase/Akt pathway was mediating the activation of mTOR in response to adiponectin.

Significantly, inhibition of PI3 kinase by LY294002 blocked the activation of mTOR in response to adiponectin (Fig. 4A). Indeed, adiponectin caused a decrease in S6 phosphorylation in the LY294002-treated cells, consistent with a dominant effect of AMPK on mTOR when PI3 kinase is blocked. To confirm that adiponectin was activating the PI3 kinase/Akt pathway, we next examined the phosphorylation of Akt at both the Thr308 and Ser473 sites. Importantly, LNCaP cells have constitutive PI3 kinase/Akt pathway activation due to PTEN loss, and hence have basal Akt phosphorylation even after serum starvation (Fig. 4B). Adiponectin caused a clear increase in Akt Ser473 phosphorylation and a more marked increase in Thr308 phosphorylation, the latter site being the direct target of PDK1 (Fig. 4B). Taken together, these results show that adiponectin in LNCaP cells stimulates both the AMPK and the PI3 kinase/Akt pathways, and that the latter PI3 kinase/Akt pathway has the dominant effect on mTOR.

**Adiponectin activation of AMPK and mTOR pathways in CWR22Rv1 cells**

To further examine adiponectin signaling in prostate cancer cells that do not have constitutive PI3 kinase/Akt activation, we used CWR22Rv1 cells...
(which have intact PTEN). Similar to LNCaP cells, adiponectin stimulated AMPK activation, with a more modest increase in ACC phosphorylation (Fig. 5A). However, in contrast to the results in LNCaP cells, there was no clear increase in mTOR activity as assessed by phosphorylation of p70 S6 kinase or S6 (Fig. 5B). As a positive control, stimulation with IGF-I increased phosphorylation of p70 S6 kinase and S6.

To assess Akt activation, we again immunoblotted for both phospho-S473 and phospho-T308 in response to adiponectin. Similar to LNCaP, adiponectin stimulated a greater increase in Akt phosphorylation at T308, but the magnitude of the increase at both sites was substantially decreased relative to LNCaP (Fig. 5C and D). In contrast to Akt phosphorylation, there was no detectable change in Erk phosphorylation (Fig. 5D). Adiponectin has been previously shown to decrease Erk activation in cardiomyocytes (Shibata et al. 2004) as well as in breast cancer cells (Dieudonne et al. 2006).

Finally, to determine whether Akt activation is a general response to adiponectin, we examined HepG2 cells. Consistent with previous data, adiponectin stimulated an increase in AMPK (not shown) and ACC phosphorylation in HepG2 cells (Fig. 5E). In contrast, there was no increase in Akt phosphorylation at S473 (not shown) or T308. As a positive control in this experiment, Akt was again phosphorylated in response to adiponectin CWR22Rv1 cells. As a further control for responsiveness of the PI3 kinase/Akt pathway in the HepG2 cells, Akt phosphorylation was increased by insulin.

**Figure 5** Adiponectin activation of AMPK and mTOR pathways in CWR22Rv1 and HepG2 cells. (A and B) Serum-starved CWR22Rv1 cells were treated with adiponectin (ADPN, 1 or 5 μg/ml) or AICAR for 15 and 30 min, and then immunoblotted for pAMPK, AMPK, pACC, ACC, pS6 kinase, pS6, total S6, and tubulin as a protein-loading control as indicated. (C and D) Serum-starved CWR22Rv1 cells were treated with adiponectin (ADPN) for 30 min and then immunoblotted as indicated. (E) HepG2 and CWR22Rv1 cells were treated with ADPN (10 μg/ml) and insulin (10 ng/ml) as indicated for 30 min, and lysates were then immunoblotted for pACC, pAkt, and total Akt as a protein-loading control.

**Discussion**

The signaling pathways downstream of adiponectin receptors are not yet completely understood, but studies in metabolically responsive cells have shown activation of AMPK as well as p38 MAP kinase and PPARα, resulting in increased fatty acid oxidation and increased insulin sensitivity (Yamauchi et al. 2001, Yoon et al. 2006). In addition, some studies have also shown activation or inactivation of Erk1/2 (Shibata et al. 2004, Luo et al. 2005a, Dieudonne et al. 2006, Luo et al. 2006, Ogunwobi & Beales 2006), IκB-α-NFκB (Ouchi et al. 2000, Tsao et al. 2002, Ogunwobi & Beales 2006), c-Jun NH(2)-terminal kinase, and signal transducer and activator of transcription 3 (Luo et al. 2005a, Miyazaki et al. 2005). While the effects on the latter pathways remain to be established and may be cell type specific, the majority of data support the conclusion that AMPK is activated downstream of adiponectin receptors in myocytes and hepatocytes (Yamauchi et al. 2002), adipocytes (Wu et al. 2003), pancreatic β cells (Huypens et al. 2005), cardiomyocytes (Shibata et al. 2004, Li et al. 2006), and endothelial cells (Chen et al. 2003, Ouchi et al. 2004). Recently, this has also been confirmed in MCF-7 breast cancer cells (Dieudonne et al. 2006). We similarly found here that adiponectin activates AMPK and increases phosphorylation of one of its major downstream targets, ACC, in prostate (LNCaP and CWR22Rv1) and HepG2 cell lines. Similarly to previous studies, activation of AMPK was rapid and transient, with the effects being gone within 30–60 min. These results indicate that AMPK
activation may constitute a general intracellular response to adiponectin. Whether LKB1, a 50 kDa serine/threonine kinase upstream of AMPK, is required for adiponectin-induced AMPK phosphorylation at Thr 172 in prostate or other cell lines remains to be determined. One recent study suggest that this could be the case, as adiponectin or AICAR-stimulated AMPK phosphorylation at Thr 172 was inhibited by over-expression of a dominant-negative (DN) LKB1 in C2C12 myotubes (Imai et al. 2006).

AMPK has been previously linked to inhibition of the mTOR pathway (Cheng et al. 2004, Luo et al. 2005b), which is activated in many tumors and by PTEN loss in a large fraction of prostate cancers. To test the hypothesis that adiponectin-mediated activation of AMPK may inhibit mTOR in prostate cancer cells, we immunoblotted for phosphorylation of p70 S6 kinase. Surprisingly, we found that the phosphorylation of p70 S6 kinase, as well as phosphorylation of S6, was increased by adiponectin treatment. This increase was blocked by rapamycin, confirming that it reflected mTOR activation, and was mediated through activation of the PI3 kinase/Akt pathway. The adiponectin-mediated activation of Akt was also observed in PTEN intact CWR22Rv1 prostate cancer cells, but was much weaker in these cells and did not result in substantial mTOR activation. In contrast, adiponectin did not activate Akt or mTOR in HepG2 cells.

Two previous studies, one in bovine aortic endothelial cells (Chen et al. 2003) and the other in human umbilical vein endothelium cells (Ouchi et al. 2004), similarly to our results, showed that adiponectin (10 μg/ml) promoted both the phosphorylation of AMPK and Akt, with both being necessary to stimulate adiponectin-induced endothelial nitric oxide synthesis and angiogenesis (Ouchi et al. 2004). Transduction with either a DN AMPK or DN Akt abolished adiponectin-induced eNOS phosphorylation. Interestingly, DN AMPK also inhibited adiponectin-induced Akt phosphorylation, while the DN Akt or pretreatment with a PI3 kinase inhibitor (LY294002 or wortmannin) blocked adiponectin-stimulated Akt and eNOS phosphorylation, but did not alter AMPK phosphorylation (Ouchi et al. 2004). These observations suggested that adiponectin-induced AMPK activation in endothelium was upstream of Akt (Ouchi et al. 2004). In contrast to this data and our data in prostate, adiponectin alone did not stimulate Akt in a serum-free environment in adipocytes (Wu et al. 2003), C2C12 myotubes (Mao et al. 2006), or in cultured bone/osteoprogenitor cells (Shinoda et al. 2006). However, adiponectin can enhance the insulin-induced phosphorylation of Akt in C2C12 myotubes (Mao et al. 2006) and in cultured bone/osteoprogenitor cells (Shinoda et al. 2006), and our preliminary data indicate that adiponectin can similarly sensitize HepG2 cells to insulin (data not shown).

The mechanisms underlying adiponectin’s action are not entirely understood. A step forward in this direction has recently been made by Mao et al. (2006) by identifying the first AdipoR1/R2 interacting protein, adaptor protein containing pleckstrin homology domain (APPL1). This 710 aa protein is localized in a subpopulation of endosomes and is highly expressed in skeletal muscle, heart, ovary, and pancreas, but it has been detected in many other human tissues (Du & Tsichlis 2005). It has been previously shown that APPL1 interacts with the small GTPase Rab5 (Miaczynska et al. 2004), the kinase domains of Akt1 and Akt2, and with the catalytic subunit of type 1A PI3K (p110α) (Mitsuuchi et al. 1999). In addition, recent studies have found that APPL1 associates with androgen receptor in prostate cancer cells and that overexpression of APPL1 enhances Akt activation by IGF-I through interaction between the APPL1 and the p85 regulatory subunit of PI3K (Yang et al. 2003). Similarly, Mao et al. (2006) found that APPL1 enhanced Akt activation by insulin, and that APPL1 mediated adiponectin signaling through AMPK and p38 MAP kinase in C2C12 myotubes. The authors also demonstrated that knocking down APPL1 by siRNA reduced and APPL1 overexpression enhanced insulin-induced Akt phosphorylation in C2C12 myotubes. Moreover, although adiponectin alone did not have an effect on Akt, there was a synergistic effect when cells were treated with both adiponectin and insulin; this synergistic effect of adiponectin and insulin-stimulated Akt phosphorylation in C2C12 cells was reduced by downregulation of APPL1 expression by siRNA (Mao et al. 2006).

Another major finding of the study was that adiponectin stimulated the interaction between the APPL1 and the small GTPase Rab5, a key regulator of endocytosis, leading to increased GLUT4 membrane translocation and increased glucose uptake (Mao et al. 2006). Their findings suggest that APPL1 may not only mediate adiponectin signaling, but also act as a regulator of the crosstalk between adiponectin signaling and insulin signaling pathways, providing a molecular mechanism for the insulin-sensitizing function of adiponectin (Mao et al. 2006). Whether APPL1 mediates adiponectin-induced phosphorylation of Akt in endothelial cells and/or activation of the PI3 kinase/Akt pathway in LNCaP and CWR22Rv1
prostate cancer cell lines remains to be determined. The fact that in our study adiponectin induced a greater increase in Akt phosphorylation at the threonine 308 site, which is also the site on the kinase domain of Akt that has been shown to interact with PDK1, and is the direct site phosphorylated by PDK1, may favor this hypothesis.

In summary, our results demonstrate adiponectin activation of both AMPK and Akt in LNCaP and CWR22Rv1 prostate cancer cells, and subsequent activation of mTOR in the LNCaP cells. The novel finding that adiponectin activation of the PI3 kinase/Akt/mTOR pathway is dominant over the AMPK-mediated inhibition of mTOR in LNCaP cells is consistent with PTEN loss in these cells and subsequent amplification of PI3 kinase signaling, and suggests that adiponectin could in some cases stimulate tumor growth through the PI3 kinase pathway. However, while this study examined the acute effects of adiponectin on PI3 kinase and AMPK pathways in serum-depleted medium, the longer-term downstream direct or indirect effects of adiponectin on cell growth in vitro and in vivo were not addressed. Indeed, a recent study found that low concentrations of HMW adiponectin could suppress the growth of prostate cancer cell lines (including LNCaP) after 5 days in serum-free medium, which could reflect dominance of the AMPK pathway after longer-term treatment, or effects on other pathways (Bub et al., 2006). It should also be noted that rapamycin and related mTOR inhibitors have limited efficacy in prostate cancer (although they may be useful in combination with other agents), so that any anti-tumor effects of elevated adiponectin in vivo may be mediated by multiple mechanisms, for example, by altering systemic levels of other hormones. In any case, further studies of adiponectin signaling in diverse normal and neoplastic cells will clearly be needed to understand how levels of this hormone may influence tumor development and stimulate or suppress the growth of established tumors.

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