Adiponectin-Activated AMPK Stimulates Dephosphorylation of AKT through Protein Phosphatase 2A Activation

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Abstract

Low serum levels of adiponectin are a high risk factor for various types of cancer. Although adiponectin inhibits proliferation and metastasis of breast cancer cells, the underlying molecular mechanisms remain obscure. In this study, we show that adiponectin-activated AMPK reduces the invasiveness of MDA-MB-231 cells by stimulating dephosphorylation of AKT by increasing protein phosphatase 2A (PP2A) activity. Among the various regulatory B56 subunits, B56γ was directly phosphorylated by AMPK at Ser299 and Ser336, leading to an increase of PP2A activity through dephosphorylation of PP2Ac at Tyr307. We also show that both the blood levels of adiponectin and the tissue levels of PP2A activity were decreased in breast cancer patients and that the direct administration of adiponectin into tumor tissues stimulates PP2A activity. Taken together, these findings show that adiponectin, derived from adipocytes, negatively regulates the invasiveness of breast cancer cells by activating the tumor suppressor PP2A. [Cancer Res 2009;69(9):4018–26]

Introduction

Obesity is an independent risk factor for the development of breast cancer. Increasing evidence suggests that adipose tissue may play an important role in regulating breast cancer progression and distant metastasis (1). The aberrant production of adipokines, adipose tissue secreting cytokines, plays an important role in the pathogenesis of obesity-related breast carcinogenesis. Some adipokines, including leptin, heparin-binding epidermal growth factor, and collagen VI act directly on breast cancer cells to stimulate their proliferation, invasiveness, and malignancy (2). Several recent studies have shown that low serum adiponectin is highly associated with obesity-related cancers, including endometrial, breast, prostate, and gastric cancers. In addition, breast tumors in women with low serum adiponectin levels are more likely to show an aggressive phenotype and adiponectin suppresses the metastasis of breast cancer cells (3).

Many reports show that inactivation of AKT by dephosphorylation, which plays a key role in tumor suppression, occurs concomitantly with the activation of AMPK. For example, the two AMPK-activating drugs, phenformin and 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), inactivate AKT by dephosphorylation (4). A mammary carcinogenesis inhibitor, 2-deoxyglucose, also activates AMPK and inactivates AKT in MDA-MB-468 human breast cancer cells (5). Deguelin, a lung cancer chemopreventive agent, induces activation of AMPK and inactivation of AKT (6). Although many reports show concomitant AMPK activation and AKT inactivation, little is known about how the activated AMPK is able to inactivate AKT.

Protein phosphatase 2A (PP2A) is a major cellular serine/threonine phosphatase that plays important roles in cell proliferation and cell transformation (7–10). For example, PP2A blocks entry into mitosis (11), inactivates the antiapoptotic protein Bcl-2 in Jurkat cells (12), and destabilizes the c-Myc oncoprotein by dephosphorylating Ser62 (13). The evidence of an antitumor role for PP2A was supported by the identification of alterations in PP2A subunits in human cancers. Many alterations in the gene encoding the Aβ subunit have been identified, primarily in human lung and colorectal cancers (14, 15). In addition, mutations (16) and reduced expression of the Aα subunit have been reported in human tumors (17) and in the MCF-7 breast cancer cell line (18). In addition to alterations of the A subunits, many alterations to the tumor suppressor activity mediated by the PP2A B56 subunit have been reported. A truncated B56γ1 isoform has been identified in a metastatic clone of the mouse B16 melanoma cells, BL16 (19). This truncation prevents a critical interaction between B56γ-containing PP2A and p53, resulting in a decrease in tumor suppressor activity (20). B56γ-containing PP2A also inhibits Wnt signaling through β-catenin degradation in Xenopus (21). B56α directs PP2A holoenzymes to c-Myc, resulting in a reduction of c-Myc levels (22). In this study, we examine the ability of AMPK, activated by adiponectin to activate PP2A by phosphorylation of its B56 subunit, which in turn suppresses invasiveness by inactivating AKT.

Materials and Methods

Reagents and plasmids. Plasmids encoding AMPK-DN were obtained from Dr. Juhun Ha (Kyunghee University). Plasmids encoding AMPK-WT and AMPK-CA were obtained from Dr. David Carling (MRC Clinical Sciences Centre). B56α, B56γ, and B56δ were amplified by pfu PCR from a human brain cDNA library and subcloned into the pCMV-tag2B expression vector. The S261A, S298A, S336A, and S298A/S336A B56γ1 mutants were generated using a mutation primer and subcloned into the pCMV-tag2B expression vector.

Invasion and proliferation assay. The ability of cells to migrate through Matrigel-coated filters was determined using modified 24-well Transwell cell culture chambers. Cells were seeded at a density of 1 × 10⁵ per well and treated with 20 μg/ml adiponectin for the indicated times. Cells that subsequently invaded the lower chamber of each plate were labeled with calcein-AM (Molecular Probes) and measured with a Wallace 1420 Victor3 plate reader (Perkin-Elmer) at an excitation of 485 ± 10 nm and an emission of 520 ± 10 nm. For the proliferation assay, cells were seeded in 12-well plates at a density of 1 × 10⁵ per well and subsequently
treated with 20 μg/mL adiponectin. The numbers of viable cells at different time points were determined by manually counting cells that did not stain with trypan blue dye.

Horizontal migration assay. The EZ-TAXIScan (Effector Cell Institute) was used to detect real-time horizontal migration of MDA-MB-231 cells. Adiponectin-pretreated MDA-MB-231 cells were placed into the single hole and DMEM containing 10% fetal bovine serum was placed into the contra-hole. A charge-coupled device camera was used to record the migration of the cells toward the DMEM containing 10% fetal bovine serum. Moving cells in a fixed gate were counted using the TAXIScan Analyzer.

Preparation of conditioned medium and zymography. Subconfluent cells were washed three times with serum-free medium and cultured for 24 h in the absence or presence of 20 μg/mL adiponectin. The conditioned medium was collected and clarified by centrifugation and then concentrated by a Centricon centrifugal filter (Millipore). Gelatinolytic activity in the conditioned medium was analyzed by zymography.

Immunoblot analysis. Analyses of cell lysates were done as described previously (23). Phospho-AMPK, phospho-AKT (Cell Signaling), and phospho-PP2Ac antibodies were used. Levels of phosphorylation were quantified relative to β-actin or α-tubulin.

AMPK, phosphatidylinositol 3-kinase, and PP2A phosphatase activity assay. Transfected cells were lysed and incubated with anti-PP2Ac antibody for 2 h. After the addition of 30 μL agarose A-beads, lysates were further incubated for 2 h at 4°C. Immuno precipitates were resuspended in 50 μL AMPK kinase reaction buffer containing 50 ng recombinant AMPK kinase, 0.16 μCi 32P-labeled ATP, and 250 μmol/L ATP followed by incubation for 30 min at room temperature. The reactions were analyzed by SDS-PAGE. Autoradiographs were visualized with a FLA-3000G Three-Laser Imaging System (FujiFilm). Phosphatidylinositol 3-kinase (PI3K) activity was assayed with the PISK ELISA kit (Echelon Biosciences) in accordance with the manufacturer's instructions. PP2A activity was determined using a serine/threonine phosphatase assay system in accordance with the manufacturer's protocols (Promega). Cells were briefly lysed with a phosphatase lysis buffer [20 mmol/L HEPES (pH 7.4), 10% glycerol, 0.1% NP-40, 30 mmol/L β-mercaptoethanol, 1 mmol/L EGTA] and measured for phosphatase activity using a PP2A-type specific buffer [50 mmol/L imidazole (pH 7.2), 0.2 mmol/L EGTA, 0.003% β-mercaptoethanol, 0.1 mg/mL bovine serum albumin]. Free phosphate, generated from a synthetic phosphothreonine peptide BRApTVA specific for PP2A, was quantified by measuring molybdate/malachite green/phosphate complex at 600 nm. EGTA and EDTA were included in the lysis buffer to inhibit PP2B and PP2C, respectively. Okadaic acid was used to confirm the specificity of these reaction conditions.

Subcellular fractionation. Cells were treated with 20 μg/mL adiponectin for 30 min and membrane fractions were obtained as described previously (24).

Tumor formation. Female BALB/c mice (6 weeks old; Dae Han Biolink) were subcutaneously injected with 1.2 × 10⁷ 4T1 adenocarcinoma cells in the right anterior mammary region. BALB/c mice bearing 4T1 murine mammary tumors were intratumorally injected with 100 μg recombinant adiponectin and 0.2 mg of the AMPK activator AICAR on day 14 after initial implantation of the 4T1 cells. Primary tumors were collected and subjected to further analysis 8 h after the injection.

Small interfering RNA preparation. The following small interfering RNA (siRNA) sequences were designed and purchased from Samchully Pharmaceuticals, siRNA-B56 forward 5'-CCGCCACCCGAUAUUAGGAAA-3' and reverse 5'-UUUCCCAUUAAUUCUGGCGC-3', siRNA-B56f; forward 5'-CCCGUUCACCCGACCAU-3' and reverse 5'-GGGAUGAUGUCUGGGAGGCG-3', siRNA-B56f, forward 5'-GCUCAUAAUACGGAGAAAGU-3' and reverse 5'-ACCAUCUGCGGAAUGGCGG-3', siRNA-B56f, forward 5'-GCUCCACCUUCAUGGAGG-3' and reverse 5'-GGGAUGAUGUCUGGGAGG-3', and siRNA-GFP was used as control.

Figure 1. Effects of adiponectin on the invasiveness of MDA-MB-231 cells. A, cells were plated in the top chamber of Matrigel-coated Transwell plates and incubated with 20 μg/mL adiponectin for the indicated time. Fluorescence of the migrated cells was measured after treatment with calcein-AM solution. The number of viable cells was manually counted at different time points of treatment. Mean ± SE of three independent experiments done in triplicate.* P < 0.05; ** P < 0.01 versus control. B, cells were pretreated with 20 μg/mL adiponectin for 18 h. Migrating cells were recorded with a charge-coupled device camera. Photographs were taken at the indicated time points. Bottom, number of cells that migrated for the indicated periods. C, cells were incubated in the presence of 20 μg/mL adiponectin for 24 h and reverse transcription-PCR was done for uPA and TIMP-2. D, cells were cultured for 24 h in serum-free DMEM in the absence or presence of 20 μg/mL adiponectin. Matrix metalloproteinase (MMP) activity in the culture supernatant was measured by gelatin zymography.
Subjects. Tumor and normal tissue samples were collected from patients with newly diagnosed breast cancer with the patients’ consent. Patients were surgically treated at the Young Dong Severance Hospital.

Statistical analysis. Data (mean ± SE) were statistically analyzed using an unpaired t test. \( P < 0.05 \) was considered a significant difference.

Results

Adiponectin suppresses metastasis of MDA-MB-231 cells. To determine the direct effects of adiponectin on the invasiveness of breast cancer cells, we performed invasion assays with MDA-MB-231 cells. The invasiveness of MDA-MB-231 cells was significantly reduced 24 h after treatment with adiponectin, but statistically significant inhibition of proliferation was not observed at the same time (Fig. 1A). One caveat to this study is that previous work has shown that adiponectin induces apoptosis of MDA-MB-231 cells. We therefore tested if adiponectin-mediated apoptosis is responsible for the observed decrease in invasiveness. Our data show that adiponectin induces apoptosis in only 3% of the cells (Supplementary Fig. S1) but suppresses invasiveness by 33%, indicating that a reduction in proliferation and an increase in apoptosis induction are responsible for only a small percent of the decrease in invasiveness induced by adiponectin. We next tested whether adiponectin could induce migration. The migration of cells in the presence of adiponectin was directly visualized using the EZ-TAXIscan, which monitors the real-time horizontal movement of cells. This analysis showed that the migration of adiponectin-pretreated MDA-MB-231 cells was dramatically inhibited compared with control cells (Fig. 1B).

To understand the detailed molecular events that underlie the inhibition of invasiveness by adiponectin, we examined the expression levels of metastasis-associated genes. Treatment with adiponectin greatly reduced the expression levels of the
AMPK Inhibits AKT by Increasing PP2A Activity

Adiponectin stimulates dephosphorylation of AKT through AMPK. To decipher the signal transduction pathway involved in the inhibition of invasion, changes in phosphorylation patterns of kinases were analyzed. Phosphorylation of p38 was slightly increased, whereas phosphorylation of AKT1/2 and GSK3β was significantly inhibited by adiponectin treatment (Supplementary Fig. S2). In addition, adiponectin time-dependently increased AMPK phosphorylation and decreased AKT phosphorylation (Fig. 2A). To determine whether AKT dephosphorylation is due to AMPK activation, AKT phosphorylation was examined after treatment with AICAR, an AMPK activator, and compound C, an AMPK inhibitor. Like adiponectin, AICAR stimulated dephosphorylation of AKT, whereas compound C blocked AMPK phosphorylation and AKT phosphorylation by adiponectin (Fig. 2B). These results indicate that AMPK activation is associated with adiponectin-induced AKT dephosphorylation.

To test whether adiponectin-activated AMPK inhibits AKT phosphorylation through the inhibition of PI3K (the upstream activator of the AKT signaling pathway), PI3K activity was measured in cells treated with adiponectin, AICAR, and compound C. Adiponectin and AICAR failed to inhibit PI3K activity, indicating that AMPK-induced AKT dephosphorylation is not mediated by the suppression of PI3K activity (Supplementary Fig. S3).

Adiponectin-activated AMPK increases PP2A activity. To examine whether PP2A is associated with AKT dephosphorylation, PP2A activity was measured. PP2A activity was greatly increased in cells treated with adiponectin or AICAR for 30 min and endogenous localization of PP2A was examined by confocal microscopy. Images were quantified by FV300 analysis. *, P < 0.05, **, P < 0.01 versus control. D, cells were treated with adiponectin, okadaic acid, or both. Membrane fraction was obtained 30 min after treatment and subjected to immunoblot analysis.
30 min before adiponectin treatment. Figure 3A shows that PP2A blocked the inhibitory effect of adiponectin on invasiveness. These results imply that PP2A is an important signaling molecule involved in the adiponectin-mediated suppression of invasiveness.

Because adiponectin-activated AMPK increases the activity of PP2A and the resulting PP2A causes the dephosphorylation of AKT, we examined whether AMPK and AKT directly interact with PP2A. An immunoprecipitation using an anti-PP2Ac antibody was done in lysates from cells overexpressing constitutively active AMPK and AKT. The precipitates were then analyzed by immunoblot. Phospho-AMPK and phospho-AKT were detected in the immunoprecipitates (Fig. 3B, top). To examine whether adiponectin increases the interaction between endogenous AKT and PP2Ac, a pull-down was done on adiponectin-treated cell lysates with an anti-AKT antibody. The level of PP2Ac in the precipitate was then determined. Adiponectin increased the interaction between endogenous AKT and PP2Ac, which could be inhibited with okadaic acid (Fig. 3B, bottom).

**Adiponectin regulates membrane localization of PP2A activity.** Because PP2A function is regulated by localization, PP2A localization was examined. Adiponectin treatment leads to the translocation of PP2A from the nucleus to the cytosol and plasma membrane, whereas PP2A remains in the nucleus with okadaic acid treatment (Fig. 3C). This result indicates that the translocation of PP2Ac to the cytosol may substantially contribute to the enhancement of the AKT dephosphorylation caused by adiponectin.

To further characterize the effect of adiponectin on the differential localization of PP2A, membrane fractions were examined in cells treated with adiponectin in the presence or absence of okadaic acid. The treatment of adiponectin markedly increased membrane localization of PP2A. However, treatment with adiponectin and okadaic acid prevented the adiponectin-induced membrane localization of PP2A (Fig. 3D).

**AMPK stimulates the dephosphorylation of the PP2A catalytic subunit leading to increased activity.** Phosphorylation of Tyr307 on PP2Ac decreases its phosphatase activity (25). Therefore, it was expected that adiponectin-activated AMPK might stimulate the dephosphorylation of PP2Ac at Tyr307, resulting in an increase of phosphatase activity. To test this possibility, the level of

![Figure 4. AMPK activates phosphatase activity of PP2A through the dephosphorylation of PP2A at Tyr307. A. cells were treated with 20 μg/mL adiponectin, 250 μmol/L AICAR, adiponectin, and 250 nmol/L okadaic acid for 30 min, and phosphorylation levels of PP2A, AKT, and AMPK were measured using immunoblot analysis. B. cells were transfected with 100 nmol/L siRNA for each of the B56 isoforms and then treated with 20 μg/mL adiponectin for 30 min. Cell lysates were subjected to immunoblot analysis. A and B, mean ± SE of three independent experiments. *, P < 0.05; **, P < 0.01, comparison of treatment with adiponectin to control or as indicated otherwise. C. following transfection with siRNAs for each B56 isoform, PP2A activity was measured 30 min after treatment with adiponectin. Mean ± SE of three independent experiments. **, P < 0.01, comparison of treatment with adiponectin to control.]
phospho-PP2Ac was measured in adiponectin-treated cells. As expected, adiponectin stimulated the dephosphorylation of PP2Ac. Cotreatment of cells with okadaic acid and adiponectin inhibited dephosphorylation of PP2Ac and also blocked AKT dephosphorylation, but AMPK activation was not affected by the phosphorylation status of PP2Ac (Fig. 4A). These results indicate that adiponectin-induced dephosphorylation of PP2Ac increases PP2A phosphatase activity, resulting in dephosphorylation of AKT.

Adiponectin-mediated increase in phosphatase activity is impaired by the knockdown of B56γ, B56γ, and B56δ isoforms of PP2A. PP2A is a trimer composed of a catalytic C subunit, a scaffold A subunit, and multiple regulatory B subunits that are thought to influence enzyme activity, substrate specificity, and subcellular localization. It is therefore possible that AMPK regulates PP2A activity through modification of the regulatory B subunit. Among the B subunits, the B56 subunit is known to play an antioncogenic role in cancer (26), so we determined which B56 isoform was related to adiponectin-mediated PP2A activation. siRNAs to various B56 isoforms were designed, and their efficiency was determined by reverse transcription-PCR (Supplementary Fig. S4). Next, cells were treated for 30 min with adiponectin after the transfection of each B56 isoform siRNA. Adiponectin-mediated AMPK phosphorylation was not impaired in the absence of all B56 isoforms, whereas adiponectin-mediated dephosphorylation of AKT and PP2Ac was significantly impaired in the absence of B56γ and B56δ expression (Fig. 4B).

To examine if impaired phosphorylation by PP2Ac also affected its activity, phosphatase activity was measured after treatment with adiponectin in cells treated with siRNA to the B56 isoforms. Knockdown of B56γ and B56δ showed no resulting increase in PP2A activity with adiponectin, whereas knockdown of B56α and B56δ expression did not affect adiponectin-increased PP2A activity. Knockdown of B56δ and B56 expression decreased even basal PP2A activity (Fig. 4C). This result implies that B56γ and B56δ expression is necessary for adiponectin-mediated PP2A activation.

AMPK directly phosphorylates B56 isoforms. We hypothesized that AMPK may phosphorylate the B56γ and B56δ isoforms, which in turn stimulate PP2A activity. To test this possibility, AMPK phosphorylation sites on the B56 isoforms were predicted using a the NetPhos 2.0 server. Several AMPK consensus sequences (27) were present in B56γ, B56δ, and B56δ, whereas the B56α subunit does not have an AMPK phosphorylation site. The B56β subunit has two predicted potential sites, and the B56γ and B56δ subunits have three sites (Supplementary Table S1). To determine whether these predicted sites are indeed phosphorylated by AMPK, overexpressed FLAG-tagged B56γ was immunoprecipitated and incubated with recombinant AMPK and isotope-labeled ATP. As predicted, B56γ was phosphorylated by AMPK (Fig. 5A). To predict the exact phosphorylation site of B56γ, S261A, S298A, and S336A mutants were generated and subjected to an in vitro phosphorylation assay. S298A and S336A mutants of B56γ were partially phosphorylated. Thus, a S298A/S336A double mutant was generated. The level of phosphorylation in the S298A/S336A double mutant was completely blocked, indicating that AMPK phosphorylates both S298 and S336 residues (Fig. 5B). To determine if PP2A activity is affected by B56γ mutants, PP2A activity was measured in cells overexpressing B56γ mutants. Expression of the S298A/S336A double mutant failed to increase phosphatase activity compared with expression of the wild-type form (Fig. 5C).

Adiponectin increases PP2A activity in vivo. We wondered whether adiponectin levels and PP2A activities were decreased in human breast tumor tissues. The level of adiponectin was significantly decreased in the serum of patients (Fig. 6A), and PP2A activities were dramatically reduced compared with that of adjacent normal tissues (Fig. 6B). Next, we wondered whether the direct administration of adiponectin into a tumor mass would increase PP2A activity in a murine mammary tumor model. Thus, intratumoral administration of adiponectin was done 14 days after initial implantation of 4T1 murine mammary tumor cells into syngeneic BALB/c mice. PP2A activity within the adiponectin-injected tumor mass was measured 8 h after the intratumoral administration. PP2A activity increased within the adiponectin-injected tumor masses (Fig. 6C), consistent with the result seen in the breast cancer cell line. The phosphorylation levels of PP2Ac and AMPK were also evaluated. The phosphorylation level of PP2Ac
decreased and the phosphorylation level of AMPK increased in adiponectin-injected tumor mass (Fig. 6D), indicating that adiponectin was indeed able to increase PP2A activity through AMPK activation in vivo.

Discussion

Although it has not yet been determined if the decreased production of adiponectin is a cause or a result of breast cancer growth, it is conceivable that tumor cells may grow well and gain metastatic potential more easily in an environment in which adiponectin is decreased because adiponectin suppresses the metastasis and proliferation of cancer cells through AKT inactivation. However, the molecular mechanism underlying this process is not fully understood. Recently, it was shown that AMPK, activated by energy depletion, phosphorylates IRS-1 at Ser\(^{794}\), leading to the inhibition of AKT through suppression of PI3K (28). In this study, however, our data clearly showed that AMPK activates PP2A, which directly inactivates AKT by the dephosphorylation of Thr\(^{308}\) and Ser\(^{473}\) without affecting PI3K activity, indicating that AMPK is able to suppress AKT function by either an IRS-1-dependent or a PP2A-dependent pathway. In addition to AKT suppression by AMPK-activated PP2A, there is another possible mechanism by which AMPK affects AKT activity. The mammalian target of rapamycin complex (mTORC) 2 phosphorylates AKT at Ser\(^{473}\) (29, 30), and the TSC2 complex is required for AKT phosphorylation by mTORC2 (31). Because AMPK phosphorylates and activates TSC2, it is possible that adiponectin-activated AMPK affects the activity of AKT through the regulation of mTORC2 activation. Therefore, it remains to be determined if adiponectin-activated AMPK is able to regulate mTORC2 activity through TSC2 phosphorylation.

In contrast to the AMPK-mediated regulation of AKT activity, AKT is also able to regulate AMPK function. AKT increases the production of ATP through accelerated aerobic glycolysis in tumors (32, 33), leading to the increase in intracellular ATP level, which in turn decreases AMPK activity. Moreover, AKT and AMPK reciprocally regulate mTORC1 activity through the differential phosphorylation of TSC2. In the presence of growth factors, AKT phosphorylates TSC2, leading to an increase in mTORC1 activity, resulting in the activation of the ATP-consuming pathway. The bidirectional communication between AMPK and AKT plays an important role in the regulation of cellular energy balance. Therefore, the disruption of AMPK and AKT crosstalk may play a critical role in the deregulation of cell cycle or tumor progression. The complete understanding of bidirectional communication between AMPK and AKT may uncover a potential therapeutic target.

Our data show that the phosphorylation of B56\(\gamma\) by AMPK increases dephosphorylation of PP2Ac at Tyr\(^{307}\), leading to the up-regulation of PP2A activity. This result may be explained by the autodephosphorylation of PP2A (25, 34). That is, AMPK-mediated
phosphorylation of B56γ affects phosphotyrosyl phosphatase activity, causing an increase in the basal level of protein tyrosine phosphatase activity of PP2A (35–38). However, we cannot completely exclude the possibility that phosphorylated B56 recruits a tyrosine phosphatase or phosphotyrosyl phosphatase activator into the entire PP2A complex, which is responsible for the dephosphorylation of PP2A at Tyr307. A complete picture of the mechanism of PP2A autodephosphorylation remains to be elucidated. In addition to the regulation of PP2A activity by the modification of catalytic subunits, it has also been reported that PP2A activity can be regulated by the modification of regulatory subunits. Various kinases including extracellular signal-regulated kinase, protein kinase A, and calcineurin-AKII have been shown to phosphotyrosyl phosphatase activity of PP2A (35–38). However, we cannot completely exclude the possibility that phosphorylated B56 does not markedly affect adiponectin-mediated increase of PP2A activity (Fig. 4C). In addition to AMPK sites, B56γ has additional phosphorylation sites that could be simultaneously phosphorylated. Elucidating the effect of multiple phosphorylations by various kinases on B56 isomorphs will be important for understanding the physiologic role of PP2A as a signal integrator of various stimuli, including growth factors and nutrients in different cellular contexts. Several reports support the tumor-suppressive role of the B56γ isomorph. Overexpression of B56γ partially reverses the tumorigenic phenotype of lung cancer cells (26) and reduces the abundance of β-catenin protein through inhibition of APC-axin formation, leading to destabilization of the β-catenin protein (44). B56γ1- and B56γ3-containing PP2A holoenzymes also directly dephosphorylate p53 at Thr18, leading to the inhibition of cell proliferation and transformation (20). The work presented here lends additional support to a tumor-suppressive role of B56γ by showing that adiponectin-activated AMPK phosphorylates B56γ, leading to the reactivation of the tumor suppressor PP2A, which in turn reduces the metastasis of breast tumor cells.

In summary, we show for the first time that adiponectin-activated AMPK directly phosphorylates a B56γ regulatory subunit, leading to the activation of PP2A, and that AMPK-activated PP2A is required to suppress AKT. Therefore, we propose that reactivation of PP2A tumor suppressor activity by adiponectin is a promising novel therapeutic strategy for treating cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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