Adiponectin Increases Skeletal Muscle Mitochondrial Biogenesis by Suppressing Mitogen-Activated Protein Kinase Phosphatase-1

Liping Qiao,[1] Brice Kinney,[1] Hyung sun Yoo,[1] Bonggi Lee,[1] Jerome Schaack,[2] and Jianhua Shao[1]

Adiponectin enhances mitochondrial biogenesis and oxidative metabolism in skeletal muscle. This study aimed to investigate the underlying mechanisms through which adiponectin induces mitochondrial biogenesis in skeletal muscle. Mitochondrial contents, expression, and activation status of p38 mitogen-activated protein kinase (MAPK) and PPARγ coactivator 1α (PGC-1α) were compared between skeletal muscle samples from adiponectin gene knockout, adiponectin-reconstituted, and control mice. Adenovirus-mediated adiponectin and MAPK phosphatase-1 (MKP1) overexpression were used to verify the relationship of MKP1 and PGC-1α in adiponectin-enhanced mitochondrial biogenesis using cultured C2C12 myotubes and PGC-1α knockout mice. An inhibitory effect of adiponectin on *MKP1* gene expression was observed in mouse skeletal muscle and cultured C2C12 myotubes. Overexpression of MKP1 attenuated adiponectin-enhanced mitochondrial biogenesis, with significantly decreased PGC-1α expression and p38 MAPK phosphorylation. Although in vivo adiponectin overexpression reduced MKP1 protein levels, the stimulative effects of adiponectin on mitochondrial biogenesis vanished in skeletal muscle of PGC-1α knockout mice. Therefore, our study indicates that adiponectin enhances p38 MAPK/PGC-1α signaling and mitochondrial biogenesis in skeletal muscle by suppressing MKP1 expression. ***Diabetes*** **61:1463–1470, 2012**

Owing to huge tissue mass and relatively high energy demand, skeletal muscle plays a critical role in energy expenditure in humans and most rodents (1,2). The mitochondrion is an organelle in mammalian cells that converts nutritional metabolites into adenosine triphosphate for cellular energy supply. It has been well documented that mitochondrial dysfunction, particularly impaired oxidative metabolism, in skeletal muscle is closely associated with obesity and insulin resistance (3–6).

Adiponectin was initially identified as an adipocyte-derived hormone that regulates energy homeostasis by increasing insulin sensitivity (7,8). Several recent studies have demonstrated that skeletal muscle also expresses adiponectin (9–12). Most importantly, regardless of the origin of adiponectin, significant evidence suggests that adiponectin improves skeletal muscle oxidative metabolism through its own receptors and downstream signaling (7,13–15). Adiponectin mRNA and blood protein levels are inversely associated with obesity (7), which is a common cause of insulin resistance in humans. Therefore, it has been proposed that hypoadiponectinemia and impaired adiponectin signaling may contribute to the decreased skeletal muscle oxidative metabolism in obesity-associated insulin resistance and even type 2 diabetes.

Using adiponectin gene knockout and transgenic mouse models, studies have demonstrated that adiponectin increases mitochondrial biogenesis and oxidative capacity in skeletal muscle (16,17). Activated AMP-activated protein kinase and increased peroxisome proliferator–activated receptor γ coactivator 1α (PGC-1α) have been suggested to mediate the regulatory effects of adiponectin on mitochondrial biogenesis and function (13,16–18). However, the signaling pathway from adiponectin receptors to *PGC-1α* gene expression is still largely unknown.

p38 is a member of the mitogen-activated protein kinase (MAPK) family and has been identified as a downstream molecule in the adiponectin-signaling pathway (15,18,19). There are four isoforms of p38 MAPK (α, β, γ, and δ), and p38α and p38γ are most abundantly expressed in skeletal muscle (20,21). p38 MAPK plays an important role in maintaining skeletal muscle energy homeostasis, myotube differentiation, and skeletal muscle tissue mass (20–23). Activation of p38 MAPK increases not only *PGC-1α* gene expression but also PGC-1α activity (24–27). Inhibition of p38 MAPK completely blocks Ca^{2+}-induced *PGC-1α* gene expression (28). Studies have also reported that adiponectin increases *PGC-1α* expression followed by mitochondrial biogenesis and fatty acid oxidation (16–18). Therefore, p38 MAPK may play a pivotal role in mediating adiponectin-stimulated *PGC-1α* gene expression, activation, and mitochondrial biogenesis in skeletal muscle. Identifying how adiponectin activates p38 MAPK will provide important information regarding the underlying mechanism through which adiponectin induces *PGC-1α* expression and mitochondrial biogenesis in skeletal muscle.

MAPK phosphatases (MKPs) are a family of protein phosphatases that specifically dephosphorylate the Thr and Tyr residues, also known as TXY motif, of MAPKs, leading to the deactivation of MAPKs (29,30). Therefore, MKPs play a critical role in regulating the activity of MAPKs. Similar to other MAPKs, the level of phosphorylation of p38 MAPK at Thr180 and Tyr182 is determined by the balance of the activities of MAPK kinases (MKKs) and MKPs.

Here, we provide evidence that MKP1 plays an important role in mediating adiponectin-enhanced mitochondrial biogenesis and oxidative metabolism. Our study demonstrated that adiponectin suppresses MKP1 protein expression in skeletal muscle, thereby leading to p38 MAPK activation and *PGC-1α* gene expression.

From the [1]Department of Pediatrics, University of California, San Diego, La Jolla, California; and the [2]Department of Microbiology, University of Colorado at Denver and Health Sciences Center, Aurora, Colorado.
Corresponding author: Jianhua Shao, jishao@ucsd.edu.
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RESEARCH DESIGN AND METHODS

Acetyl-CoA, oxaloacetate, 5,5′-dithiobis (2-nitrobenzoic acid), triethanolamine, and insulin were purchased from Sigma (St. Louis, MO). Anti-MKP1 antibody was from Millipore (Billerica, MA). Antibodies for PGC-1α and phospho– and total p38 MAPK (all isoforms) were from Cell Signaling (Danvers, MA). Horse serum, penicillin-streptomycin, Dulbecco's modified Eagle's medium (DMEM), and MitoTracker Green FM were from Invitrogen (Carlsbad, CA). Recombinant mouse adiponectin protein was purchased from Biovendor (Candier, NC).

Adiponectin gene knockout (Adipoq$^{-/-}$) mice were created in Dr. Philipp Scherer's laboratory (31) on a 129/SvEv genetic background and were backcrossed to C57BL/6 for six generations. Wild-type (WT) littermates were used as controls for Adipoq$^{-/-}$ mice. *PGC-1α* gene knockout mice (*PGC-1α*$^{-/-}$) were provided by Dr. Daniel P. Kelly (Washington University, St. Louis, MO) (32). All mice were maintained under standardized conditions with a 12-h/12-h light/dark cycle. The experiments using mouse models were carried out under the Association for Assessment and Accreditation of Laboratory Animal Care guidelines with approval of the Animal Care and Use Committee. To overexpress or reconstitute adiponectin, we used adenovirus-Acrp30 (Ad-Acrp30) vector–mediated intravenous gene transduction, which leads to transduced gene expression primarily in hepatocytes (33–35). For these studies, purified adenovirus (1×10^9 pfu per mouse) vector was injected into the mouse through the tail vein (35). Tissue samples were collected 3 days after viral injection. Consistent with our previous report (34), mouse blood adiponectin levels were increased ~10- to 12-fold compared with WT mice that received adenovirus encoding green fluorescent protein (GFP) (Supplementary Fig. 1*A*). In addition, blood multimeric adiponectin levels were proportionally elevated in Ad-Acrp30–injected mice (Supplementary Fig. 1*B*) (34).

Cell culture. C2C12 myoblasts were purchased from American *Type Culture* Collection. Immortalized mouse embryonic fibroblasts (MEFs) from *MKK3* and *-6* double knockout or WT mice were established by the laboratory of Dr. Roger Davis (University of Massachusetts Medical School, Worcester, MA). C2C12 cells were maintained in DMEM supplemented with 10% (Gemini Bio-Products, Woodland, CA), 200 mmol/L L-glutamine, and 200 units/mL penicillin and 50 ug/mL streptomycin in an atmosphere of 95% air and 5% CO_2. When C2C12 cells reached 90% confluence, myotube differentiation was induced by switching the medium to DMEM supplemented with 2% horse serum. The culture medium was changed daily. Polynucleated myotubes were obtained within 4 days. A coculture system was used for adiponectin treatment (33). After overnight coculture, adiponectin in the medium reached a level of 40–50% of that in C57BL/6 mouse serum (Supplementary Fig. 1*C*). All multimeric adiponectins were secreted from Ad-Acrp30–transduced FAO cells and were present in the coculture medium (33) (Supplementary Fig. 1*B*). Differentiated C2C12 myotubes were cocultured overnight in an insert well with Ad-Acrp30– or Ad-GFP–transduced FAO cells.

Adenoviral vectors and plasmid construction. Adenoviral vectors encoding adiponectin, MKP1, LacZ, and GFP were created using the ViraPower Adenoviral Expression system (Invitrogen). Adenovirus was produced in HEK293 cells and purified by cesium chloride gradient centrifugation as previously described (34). Plasmids pcDNA-MKP1, pcDNA-p38, and pcDNA-PGC-1α were created by subcloning the cDNAs into pcDNA3.1 vector. Plasmid vector encoding 262A/265A/298A triple-mutated PGC-1α (PGC-1α 3A) was provided by Dr. Pere Puigserver (Dana-Farber Cancer Institute, Boston, MA). The luciferase reporter construct was generated by inserting the 2-kb mouse Cidea promoter into pGL3 basic vector (pGL3-Cidea-Luc).

Mitochondrial content and enzyme activity assay. Mitochondrial content was determined by measuring mitochondrial DNA (mtDNA) levels, mitochondrial mass with MitoTracker Green probe, and citrate synthase activity. DNA was prepared using a Qiagen DNeasy blood and tissue kit. mtDNA was quantified using real-time PCR using a pair of primers for the Cox2 mtDNA region: forward, 5′-TTTTCAGGCTTCACCCTAGATGA-3′; reverse, 5′-GAAGAATGTTATGTTTACTCCTACGAATATG-3′ (36). MitoTracker Green is a cell-permeable mitochondrion-selective dye and has been used for mitochondrial mass measurement (16). Briefly, after adiponectin treatment, C2C12 myotubes were washed twice with PBS and stained with 100 nmol/L MitoTracker Green FM for 30 min at 37°C. Fluorescence intensity was detected following the protocol provided by the manufacturer, and values were normalized to total protein levels.

Citrate synthase activity was measured spectrophotometrically using cell or tissue homogenates (16). Briefly, citrate synthase activity was measured at 37°C in 0.1 mol/L Tris-HCl (pH 8.3) assay buffer containing 0.1 mmol/L 5,5′-dithiobis (2-nitrobenzoic acid), 0.5 mmol/L oxaloacetate, 50 μmol/L EDTA, and 5 mmol/L triethanolamine hydrochloride. After an initial 2-min absorbance reading taken at 412 nm, the reaction was initiated with the addition of 3.0 mmol/L acetyl-CoA, and the change in absorbance was measured every 10 s for 2 min.

Statistical analysis. Data are expressed as means ± SEM. Statistical analysis was performed using Student *t* test or ANOVA, followed by contrast test with Tukey or Dunnett error protection. Differences were considered significant at $P < 0.05$.

RESULTS

Adiponectin induces p38 MAPK activation, *PGC-1α* expression, and mitochondrial biogenesis in skeletal muscle. Using Adipoq$^{-/-}$ mice and adenovirus-mediated in vivo gene transduction, our results show that phosphorylation of p38 MAPK at Thr180 and Tyr182, which represents its activation state, was significantly reduced in skeletal muscle of Adipoq$^{-/-}$ mice (Fig. 1*A*). Consistent with previous reports (16–18), mitochondrial content and expression levels of PGC-1α and mitochondrial marker Cox5a were significantly reduced in skeletal muscle of Adipoq$^{-/-}$ mice (Supplementary Fig. 2*A–C*). In contrast, adiponectin reconstitution increased p38 MAPK phosphorylation in skeletal muscle from mice that received Ad-Acrp30 injection (Fig. 1*A*). Furthermore, overnight adiponectin treatment also significantly increased p38 MAPK phosphorylation and PGC-1α protein levels in cultured C2C12 myotubes (Fig. 1*B*). The levels of mtDNA and Cox5α mRNA were also significantly increased in adiponectin-treated C2C12 myotubes (Supplementary Fig. 2*D* and *E*). These results clearly indicate that adiponectin increases p38 MAPK phosphorylation, PGC-1α expression, and mitochondrial biogenesis in skeletal muscle.

Adiponectin suppresses *MKP1* gene expression in skeletal muscle. MKK3 and MKK6 are two key upstream kinases for p38 MAPK activation. To verify the role of MKK3 and MKK6 in adiponectin-induced p38 MAPK activation, MEFs from *MKK3* and *MKK6* double knockout (*MKK3*&6KO) mice were used. The results show that the magnitudes of adiponectin-induced p38 MAPK phosphorylation were significantly lower in *MKK3*&6KO MEFs compared with WT MEFs (Fig. 2), which suggests that MKK3 and MKK6 are required for maximal adiponectin-stimulated p38 MAPK phosphorylation. This result is in line with a recent study, which reported that MKK3 plays an important role in adiponectin-induced p38 MAPK phosphorylation (19). However, although there was a reduction of adiponectin-induced p38 MAPK phosphorylation in *MKK3*&6KO MEFs, adiponectin treatment still significantly increased p38 MAPK phosphorylation in *MKK3*&6KO MEFs (Fig. 2). In other words, *MKK3* and *-6* gene deletion did not completely eliminate the stimulatory effects of adiponectin on p38 MAPK activation. These results suggest that, in addition to MKK3 and MKK6, other molecules or signaling pathways are involved in adiponectin-induced p38 MAPK activation in skeletal muscle.

MKP1 is the prototype of the MKP family and is ubiquitously expressed. Our results show that the expression levels of MKP1 were significantly increased in skeletal muscle of Adipoq$^{-/-}$ mice, while adiponectin reconstitution reduced MKP1 protein levels in skeletal muscle (Fig. 1*A*). Similar inhibitory effects of adiponectin on MKP1 expression were observed in C2C12 myotubes (Fig. 1*B*). These results indicate that adiponectin inhibits MKP1 expression in skeletal muscle.

Adiponectin enhances p38 MAPK phosphorylation, PGC-1α expression, and mitochondrial biogenesis by suppressing MKP1 expression in C2C12 myotubes. A previous in vivo study reported that MKP1-deficient mice expressed higher levels of PGC-1α in skeletal muscle with enhanced energy expenditure (30). Using adenovirus-mediated gene transduction and cultured C2C12 myotubes,

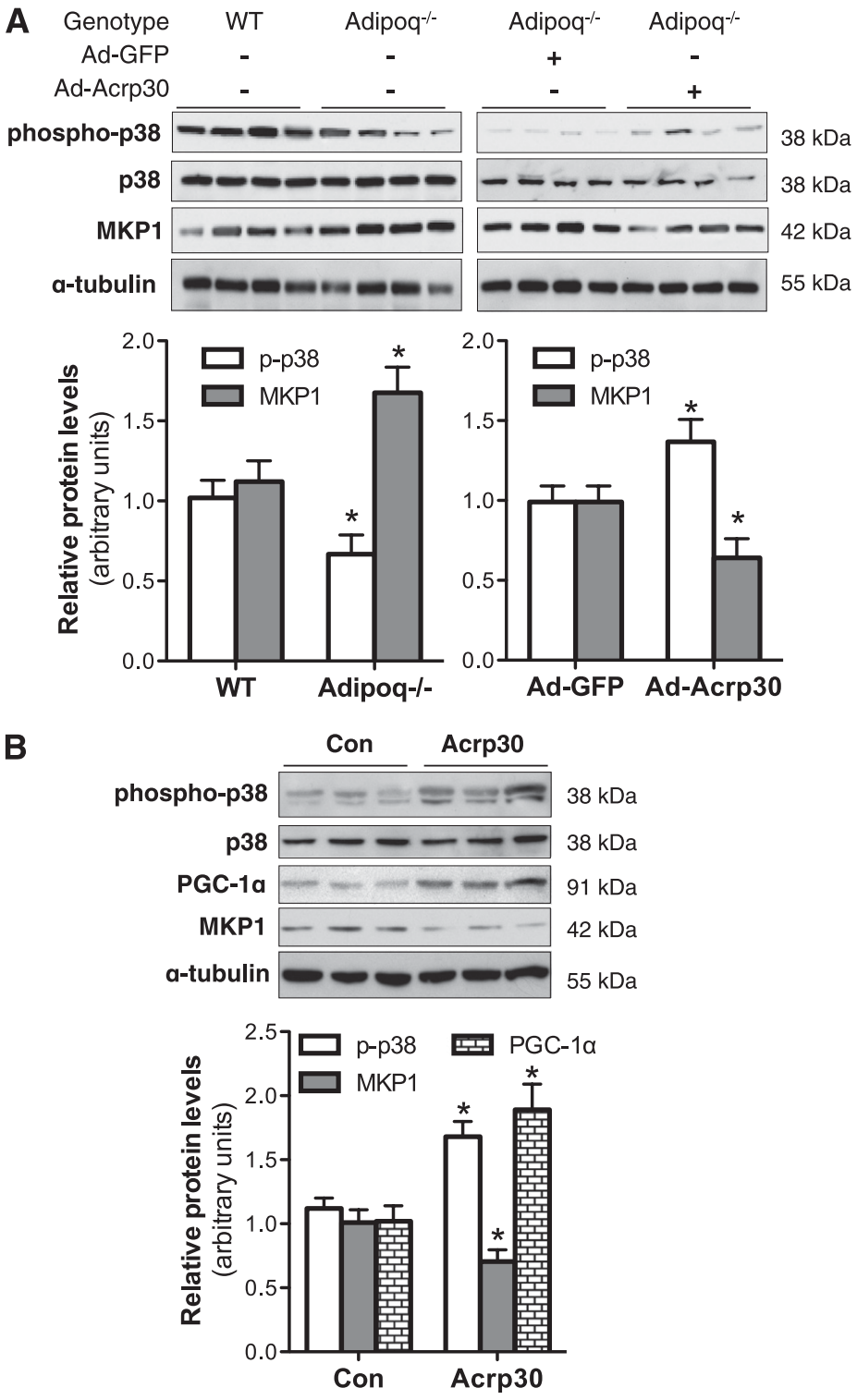

FIG. 1. Adiponectin induces p38 MAPK phosphorylation and *PGC-1α* expression but decreases MKP1 protein levels in mouse skeletal muscle and C2C12 myotubes. *A*: Protein samples were prepared from the gastrocnemius muscle of WT and Adipoq$^{-/-}$ mice. For adiponectin reconstitution, Ad-Acrp30 was injected into indicated mice through the tail vein. Ad-GFP was used as control treatment. Tissues were collected 3 days after injection with overnight fasting. Relative protein levels of phospho-p38 (p-p38) MAPK, p38 MAPK, and MKP1 were measured by Western blot; $n = 8$. *B*: Fully differentiated C2C12 myotubes were cocultured overnight with FAO cells transduced with Ad-Acrp30 or Ad-GFP (control [Con]) in DMEM without serum. Protein levels were quantified by Western blot using indicated antibodies; $n = 6$. *$P < 0.05$ vs. WT or control cells. Data are expressed as means ± SEM.

we studied the effects of MKP1 on *PGC-1α* gene expression and mitochondrial biogenesis. As expected, MKP1 overexpression robustly decreased p38 MAPK phosphorylation (Fig. 3*A*). Expression of PGC-1α was also significantly reduced at the protein (Fig. 3*A*) and mRNA (Supplementary Fig. 3) levels. Mitochondrial content and citrate synthase activities were also significantly lower in Ad-MKP1–transduced myotubes compared with Ad-GFP–treated cells

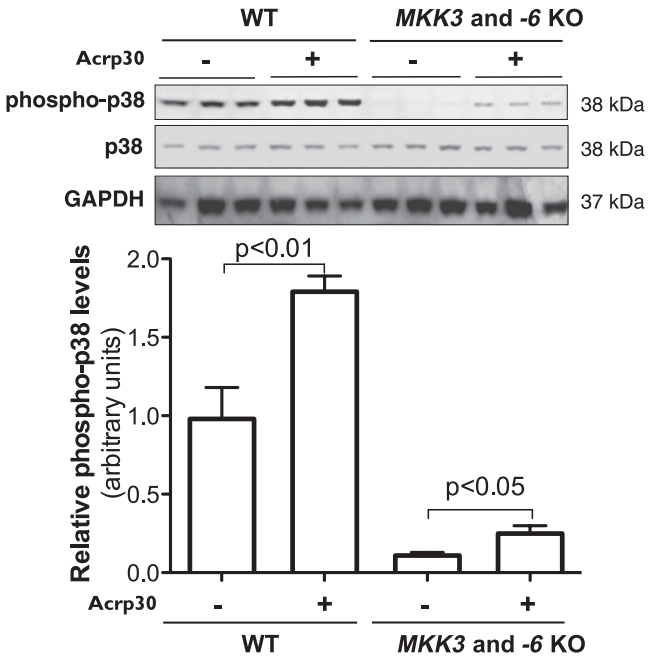

FIG. 2. Adiponectin induces p38 MAPK phosphorylation in *MKK3* and *-6* gene knockout (KO) MEFs. Immortalized MEFs from WT and *MKK3* and *-6* knockout mice were cocultured overnight with FAO cells, which were transduced with Ad-Acrp30 (+) or Ad-GFP (-). Phosphorylation and total protein levels of p38 MAPK were detected by Western blot. Quantified data represent three separate studies; $n = 6$. Data are expressed as means ± SEM. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

(Fig. 3*B* and *C*). Results from these in vitro studies further demonstrate that MKP1 plays an important role in *PGC-1α* gene expression and mitochondrial biogenesis in myotubes.

Posttranslational modification plays an important role in regulating PGC-1α activity. p38 MAPK phosphorylates Thr262, Ser265, and Thr298 residues of PGC-1α and increases its activity (25). MKP1 reduces p38 MAPK activity. We carried out a functional assay to determine whether MKP1 reduces PGC-1α activity through p38 MAPK–induced PGC-1α phosphorylation by using Cidea promoter–directed luciferase reporter construct and a plasmid encoding PGC-1α with 262A/265A/298A triple mutation (PGC-1α 3A) (25,37). Consistent with previous reports (25), p38 MAPK increased PGC-1α activity, while MKP1 displayed an opposing effect (Fig. 3*D*). However, MKP1 failed to reduce luciferase activities in cells expressing PGC-1α 3A. Together, these results indicate that MKP1 reduces PGC-1α activity by decreasing both its expression and p38 MAPK–induced phosphorylation.

For determination of the role of MKP1 in adiponectin-enhanced *PGC-1α* gene expression and mitochondrial biogenesis, MKP1 was overexpressed using an adenovirus vector to counteract the inhibitory effects of adiponectin on MKP1 expression. MKP1 protein levels were significantly elevated in C2C12 myotubes owing to the potent CMV promoter (Fig. 3*A*). Similar to the results presented in the section above, overnight adiponectin treatment significantly increased *PGC-1α* expression (Supplementary Fig. 4A) and levels of mitochondrial content and citrate synthase activity in control vector-transduced C2C12 myotubes (Fig. 3*E* and Supplementary Fig. 4*B*). However, expression of PGC-1α and mtDNA and citrate synthase activity were comparable between adiponectin-treated and control cells with MKP1 overexpression (Fig. 3*E* and Supplementary Fig. 4). These results indicate that MKP1 overexpression abolishes the stimulating effects of adiponectin on *PGC-1α* gene expression and mitochondrial biogenesis in myotubes.

MKP1 mediates adiponectin-enhanced mitochondrial biogenesis through PGC-1α. The results from the above studies suggest that adiponectin increases *PGC-1α* gene expression and mitochondrial biogenesis in skeletal muscle. Our studies and results from other studies (30) indicate that MKP1 inhibits mitochondrial biogenesis. For verification of the relationship of MKP1 and PGC-1α in adiponectin-induced mitochondrial biogenesis in skeletal muscle, the *PGC-1α*$^{-/-}$ mouse model was used. Blood adiponectin protein levels were significantly elevated after adenoviral vector–mediated in vivo gene transduction (34). Overexpression of adiponectin increased PGC-1α protein levels in skeletal muscle from WT control mice (Fig. 4*A*). The levels of citrate synthase activity (Fig. 4*B*), mitochondrial marker Cox5α, and mtDNA (Fig. 4*C* and *D*) were also significantly increased in Ad-Acrp30–treated WT mice. However, despite significantly reduced MKP1 protein levels in Ad-Acrp30–treated WT and *PGC-1α*$^{-/-}$ mice (Fig. 4*E*), adiponectin overexpression failed to increase Cox5α and citrate synthase activity in *PGC-1α*$^{-/-}$ mice (Fig. 4*B–D*). These results indicate that PGC-1α plays an essential role in adiponectin-enhanced mitochondrial biogenesis. These results further suggest that MKP1 mediates adiponectin-enhanced mitochondrial biogenesis through PGC-1α.

DISCUSSION

Adiponectin is an adipocyte-derived hormone that exhibits multiple favorable functions in glucose and lipid metabolism. In addition to its insulin-sensitizing effect, recent studies have demonstrated that adiponectin may regulate energy metabolism through pathways independent of insulin signaling. Previous studies and the current study have demonstrated that adiponectin enhances mitochondrial biogenesis. Through the use of a *PGC-1α* knockout mouse model, our study further confirms the key role of PGC-1α in adiponectin-enhanced mitochondrial biogenesis. Most importantly, our study identifies MKP1 as a new downstream molecule of adiponectin signaling. This study provides clear evidence that adiponectin reduces MKP1 protein levels leading to increased p38 MAPK and PGC-1α activation in skeletal muscle.

It has been well established that p38 MAPK plays a critical role in skeletal muscle biogenesis and energy metabolism (25–27,38). p38 MAPK is among the first group of signaling proteins that have been identified as downstream of the adiponectin signaling pathway (15,18,19). However, it was unknown for a long period how adiponectin induces p38 MAPK and its downstream activation. A recent study reported that the adaptor protein APPL1 (adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1) selectively transduces signals from the adiponectin receptor to the TGF-β-activated kinase 1 (TAK1)-MKK3-p38 MAPK pathway (19). Consistent with this report, our study found that *MKK3* and *MKK6* gene deletion significantly attenuated adiponectin-induced p38 MAPK phosphorylation. These studies indicate that the canonical MKK/MAPK pathway is required in adiponectin-induced p38 MAPK activation. Although other posttranslational modifications may exist, at this point, phosphorylation of the TXY motif has been identified as the on-and-off switch

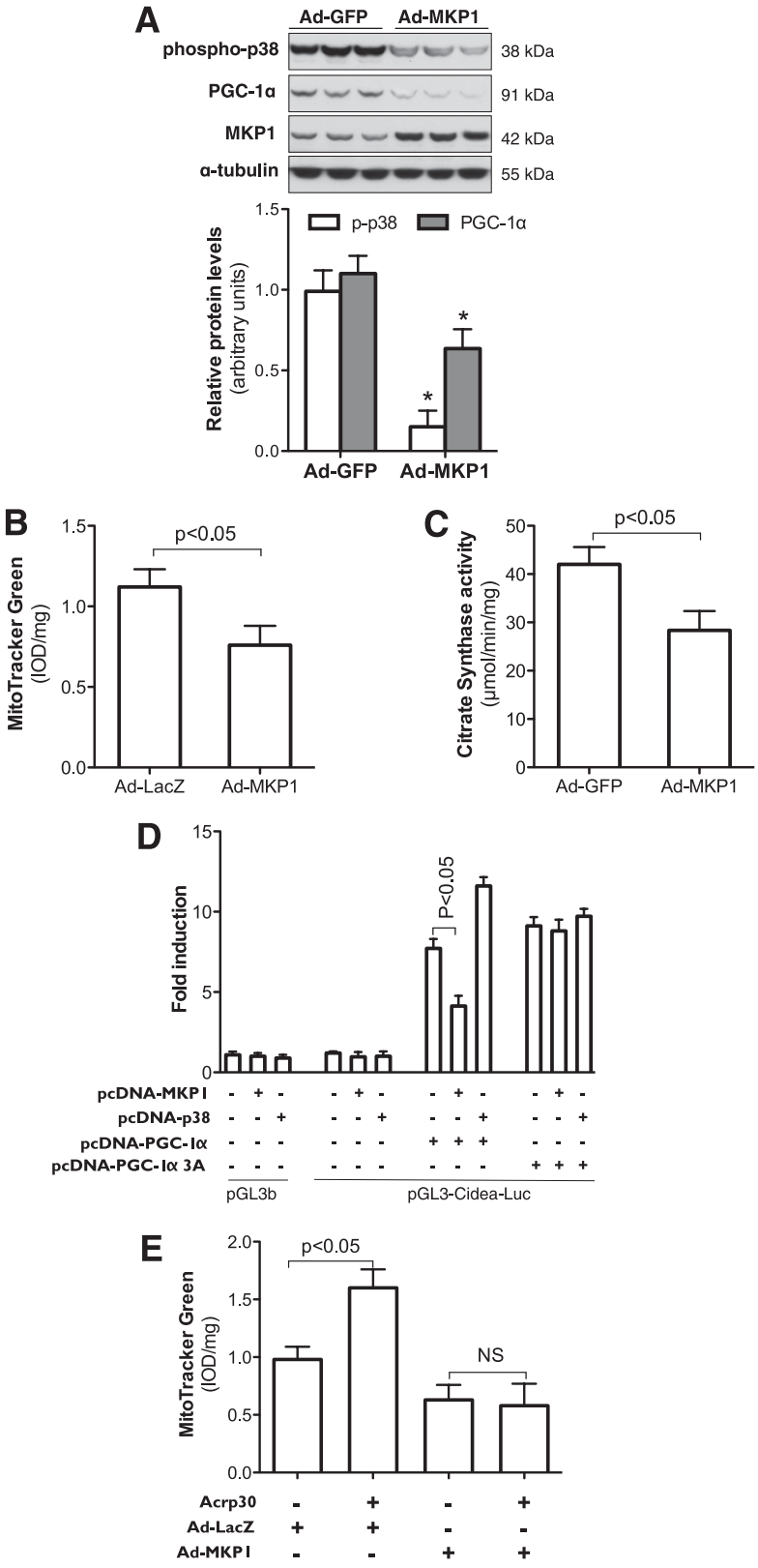

FIG. 3. Overexpression of MKP1 attenuates adiponectin-enhanced mitochondrial biogenesis in C2C12 myotubes. MKP1 was overexpressed in differentiated C2C12 myotubes using an adenovirus vector-encoding mouse MKP1 (*A–C* and *E*). Control cells were transduced with viral vector encoding GFP or LacZ, which was used to avoid interference with fluorescent readings. Twenty-four hours after adenovirus transduction, some cells were treated with adiponectin overnight using the coculture system in which adiponectin was secreted from Ad-Acrp30–transduced FAO cells (*E*). Protein levels of phospho-p38 (p-p38) MAPK, PGC-1α, and MKP1 were detected using Western blot (*A*). Mitochondrial content was measured using MitoTracker Green (*B* and *E*). Citrate synthase activity was measured using cell lysates (*C*). For *A–C* and *E*, $n = 6$, *$P < 0.05$ vs. Ad-GFP–treated cells; data are expressed as means ± SEM. For promoter-activity assay (*D*), Cidea promoter–directed luciferase constructs were transiently cotransfected into C2C12 cells with pcDNA-MKP1 or pcDNA-p38 and PGC-1α or PGC-1α 3A. Twenty-four hours after transfection, luciferase activities were measured and normalized to cotransfected LacZ activity. Fold increase of luciferase activities are presented; $n = 8$. IOD, integrated optical density.

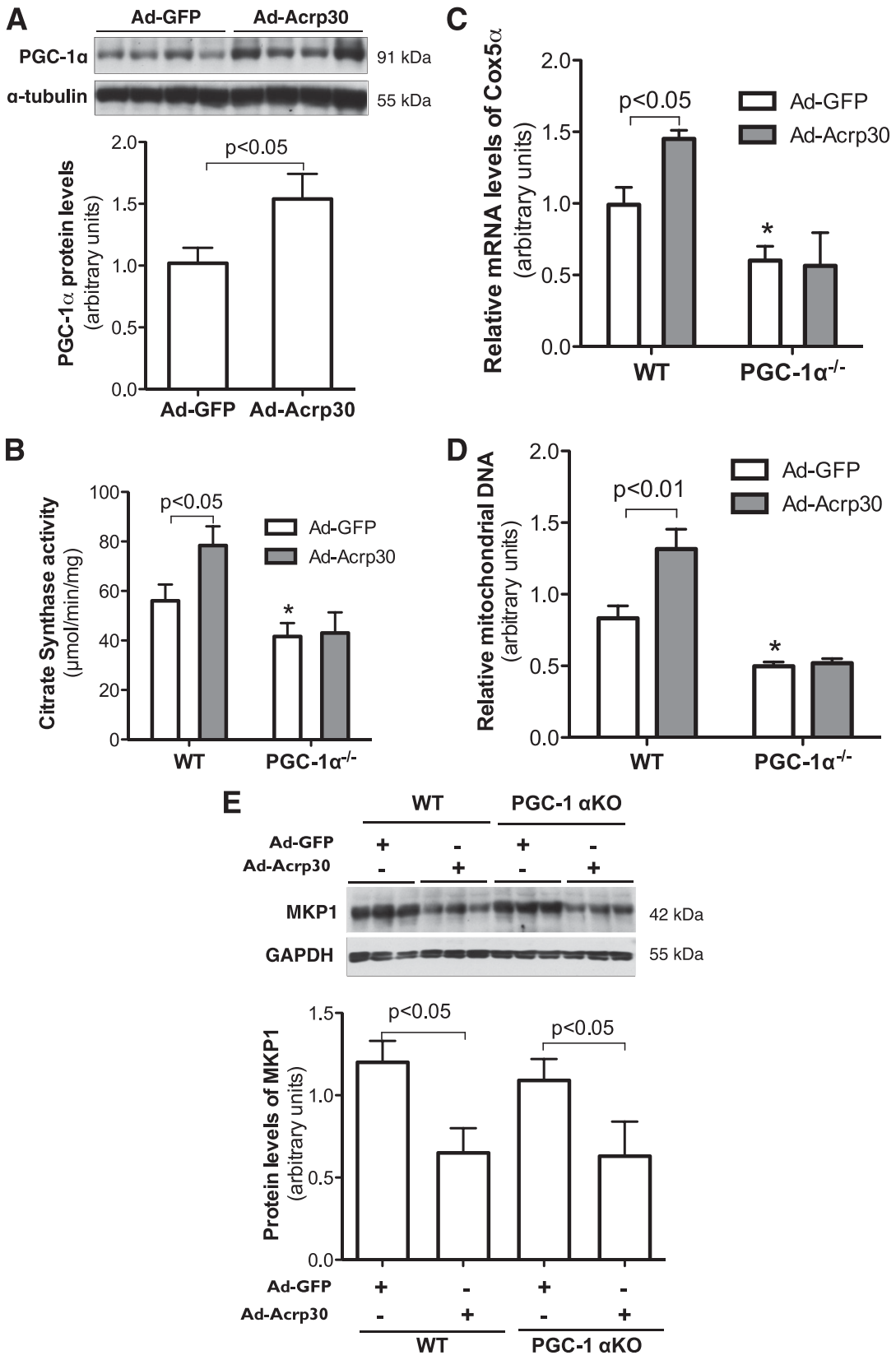

FIG. 4. Adiponectin suppresses MKP1 expression but fails to increase mitochondrial biogenesis in skeletal muscle of *PGC-1α* knockout (KO) mice. To increase the circulating adiponectin concentration, Ad-Acrp30 was injected into 8- to 10-week-old WT or *PGC-1α* knockout male mice. Three days after injection, gastrocnemius muscle samples were collected after overnight fasting. Detailed procedures for protein, citrate synthase activity, and mRNA and mtDNA measurement are described in RESEARCH DESIGN AND METHODS. Elevated PGC-1α protein levels were observed in Ad-Acrp30–treated WT mice (*A*). Correspondingly, citrate synthase activity (*B*), Cox5α mRNA (*C*), and mtDNA content (*D*) were also significantly increased in Ad-Acrp30–treated WT mice but not in Ad-Acrp30–treated *PGC-1α* knockout mice compared with their controls. However, adiponectin overexpression reduced MKP1 protein levels in skeletal muscle of both WT and *PGC-1α* knockout mice (*E*). *n* = 8, **P* < 0.05 vs. Ad-GFP–treated WT mice; data are expressed as means ± SEM.

for p38 MAPK and other MAPKs. MKK3 and MKK6 phosphorylate and activate p38 MAPK. In contrast, MKPs, including MKP1, specifically dephosphorylate the TXY motif and deactivate p38 MAPK (29). The counteraction of MKKs and MKPs determines the phosphorylation level and kinase activity of p38 MAPK. Therefore, MKPs are also important in controlling p38 MAPK activation. Using both in vivo and in vitro systems, our study demonstrates that adiponectin reduces MKP1 protein levels in skeletal muscle. Furthermore, MKP1 overexpression attenuated adiponectin-enhanced *PGC-1α* gene expression and mitochondrial biogenesis in C2C12 myotubes. Decreased MKP1 protein leads to enhanced p38 MAPK activation and then to higher PGC-1α expression and mitochondrial biogenesis in skeletal muscle.

PGC-1α is a transcriptional coactivator and plays an important role in mitochondrial biogenesis. Our study suggests that adiponectin enhances mitochondrial biogenesis through the MKP1/p38/PGC-1α pathway. p38 MAPK activates PGC-1α by increasing not only its gene expression but also phosphorylation (24–27). This raises the question of whether adiponectin increases PGC-1α activity by increasing p38-mediated phosphorylation of PGC-1α. Owing to the lack of antibody against phosphorylated PGC-1α at Thr262, Ser265, and Thr298, which are phosphorylated by p38, our study does not provide direct evidence indicating whether adiponectin increases PGC-1α phosphorylation at these three sites. However, the functional assay showed that expressing triple mutant PGC-1α 3A abolished p38 MAPK–enhanced promoter activation but not adiponectin-increased luciferase activities (Supplementary Fig. 5*A*). These results suggest that, in addition to p38-mediated PGC-1α phosphorylation, adiponectin enhances PGC-1α activity through other pathway(s).

It has been reported that phosphorylation of Ser570 of PGC-1α inhibits its transactivity (39). Using a phospho-specific antibody, we found that phosphorylation levels of PGC-1α Ser570 were remarkably decreased in adiponectin-treated C2C12 myotubes and skeletal muscle of adiponectin-overexpressed mice (Supplementary Fig. 5*B* and *C*). Apparently, AKT is not responsible for adiponectin-decreased PGC-1α phosphorylation at Ser570 because increased AKT phosphorylation was observed. Although more studies are required to identify the underlying mechanism, our study suggests that adiponectin increases PGC-1α activity by stimulating both gene expression and posttranslational modification.

MKP1 is a key negative regulator of the MAPK pathway, which plays a critical role in multiple physiological processes in skeletal muscle including mitochondrial biogenesis and energy metabolism (29,30). Low-level mitochondrial content and activity in skeletal muscle have been reported in both type 2 diabetic patients and obese subjects (3–5). Through use of mouse model and biochemical approaches, a study from Bennett's group convincingly demonstrated that high-fat diet induces *MKP1* gene expression in skeletal muscle and results in inhibition of the p38 MAPK/PGC-1α pathway and loss of oxidative myofibers (30). The same group has also reported that *MKP1* gene deletion increases skeletal muscle mitochondrial respiration (40). Hypoadiponectinemia occurs in obese human subjects and diet-induced obese mouse models (7). Our study indicates that adiponectin inhibits *MKP1* gene expression in skeletal muscle. Therefore, we postulate that hyoadiponectinemia may contribute to high-fat diet–induced *MKP1* gene expression and its adverse effects on oxidative metabolism in skeletal muscle. However, caution should be taken in applying this finding in mice to humans. More animal and human studies are required to support this possibility.

The underlying mechanism through which adiponectin suppresses *MKP1* gene expression is not clear. Previous studies have suggested that activation of p38 MAPK and Jun NH_2-terminal kinase induces *MKP1* gene expression (29), which may serve as a feedback inhibitor to prevent prolonged MAPK activation. Clearly, this is not the case for adiponectin-induced downregulation of *MKP1* gene expression in myotubes. Similar to other reports, our study showed that adiponectin stimulates p38 MAPK phosphorylation. If p38 MAPK increases *MKP1* gene expression, we should expect an increase of *MKP1* expression in adiponectin-treated myotubes. However, the opposite effect was observed in our study. On the other hand, the inverse changes of p38 MAPK activity and *MKP1* expression in adiponectin-treated myotubes suggest that adiponectin-induced p38 MAPK activation is most likely mediated by decreased *MKP1* gene expression.

In summary, our results demonstrate that activation of the p38 MAPK/PGC-1α pathway is essential in adiponectin-induced mitochondrial biogenesis and oxidative metabolism in skeletal muscle. Inhibition of *MKP1* gene expression plays an important role in adiponectin-stimulated p38 MAPK activation.

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L.Q., B.K., H.s.Y., and B.L. contributed research data. J.Sc. contributed to viral vectors and discussion. J.Sh. conceived the study and wrote the manuscript. J.Sh. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 1981;30:1000–1007
2. Katz LD, Glickman MG, Rapoport S, Ferrannini E, DeFronzo RA. Splanchnic and peripheral disposal of oral glucose in man. Diabetes 1983;32:675–679
3. Befroy DE, Petersen KF, Dufour S, et al. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. Diabetes 2007;56:1376–1381
4. Björntorp P, Scherstén T, Fagerberg SE. Respiration and phosphorylation of mitochondria isolated from the skeletal muscle of diabetic and normal subjects. Diabetologia 1967;3:346–352
5. Petersen KF, Befroy D, Dufour S, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 2003;300:1140–1142
6. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002;51:2944–2950
7. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005;26:439–451

8. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746–26749
9. Amin RH, Mathews ST, Camp HS, Ding L, Leff T. Selective activation of PPARgamma in skeletal muscle induces endogenous production of adiponectin and protects mice from diet-induced insulin resistance. Am J Physiol Endocrinol Metab 2010;298:E28–E37
10. Delaigle AM, Jonas JC, Bauche IB, Cornu O, Brichard SM. Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. Endocrinology 2004;145:5589–5597
11. Krause MP, Liu Y, Vu V, et al. Adiponectin is expressed by skeletal muscle fibers and influences muscle phenotype and function. Am J Physiol Cell Physiol 2008;295:C203–C212
12. Liu Y, Chewchuk S, Lavigne C, et al. Functional significance of skeletal muscle adiponectin production, changes in animal models of obesity and diabetes, and regulation by rosiglitazone treatment. Am J Physiol Endocrinol Metab 2009;297:E657–E664
13. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288–1295
14. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7:947–953
15. Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762–769
16. Civitarese AE, Ukropcova B, Carling S, et al. Role of adiponectin in human skeletal muscle bioenergetics. Cell Metab 2006;4:75–87
17. Iwabu M, Yamauchi T, Okada-Iwabu M, et al. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. Nature 2010;464:1313–1319
18. Yoon MJ, Lee GY, Chung JJ, Ahn YH, Hong SH, Kim JB. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. Diabetes 2006;55:2562–2570
19. Xin X, Zhou L, Reyes CM, Liu F, Dong LQ. APPL1 mediates adiponectin-stimulated p38 MAPK activation by scaffolding the TAK1-MKK3-p38 MAPK pathway. Am J Physiol Endocrinol Metab 2011;300:E103–E110
20. Ruiz-Bonilla V, Perdiguero E, Gresh L, et al. Efficient adult skeletal muscle regeneration in mice deficient in p38beta, p38gamma and p38delta MAP kinases. Cell Cycle 2008;7:2208–2214
21. Ho RC, Alcazar O, Fujii N, Hirshman MF, Goodyear LJ. p38gamma MAPK regulation of glucose transporter expression and glucose uptake in L6 myotubes and mouse skeletal muscle. Am J Physiol Regul Integr Comp Physiol 2004;286:R342–R349
22. Zetser A, Gredinger E, Bengal E. p38 mitogen-activated protein kinase pathway promotes skeletal muscle differentiation. Participation of the Mef2c transcription factor. J Biol Chem 1999;274:5193–5200
23. Röckl KS, Witczak CA, Goodyear LJ. Signaling mechanisms in skeletal muscle: acute responses and chronic adaptations to exercise. IUBMB Life 2008;60:145–153
24. Knutti D, Kressler D, Kralli A. Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor. Proc Natl Acad Sci USA 2001;98:9713–9718
25. Puigserver P, Rhee J, Lin J, et al. Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. Mol Cell 2001;8:971–982
26. Akimoto T, Pohnert SC, Li P, et al. Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. J Biol Chem 2005;280:19587–19593
27. Cao W, Daniel KW, Robidoux J, et al. p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. Mol Cell Biol 2004;24:3057–3067
28. Wright DC, Geiger PC, Han DH, Jones TE, Holloszy JO. Calcium induces increases in peroxisome proliferator-activated receptor gamma coactivator-1alpha and mitochondrial biogenesis by a pathway leading to p38 mitogen-activated protein kinase activation. J Biol Chem 2007;282:18793–18799
29. Chi H, Flavell RA. Acetylation of MKP-1 and the control of inflammation. Sci Signal 2008;1:pe44
30. Roth RJ, Le AM, Zhang L, et al. MAPK phosphatase-1 facilitates the loss of oxidative myofibers associated with obesity in mice. J Clin Invest 2009;119:3817–3829
31. Nawrocki AR, Rajala MW, Tomas E, et al. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. J Biol Chem 2006;281:2654–2660
32. Leone TC, Lehman JJ, Finck BN, et al. PGC-1alpha deficiency causes multisystem energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol 2005;3:e101
33. Qiao L, Kinney B, Schaack J, Shao J. Adiponectin inhibits lipolysis in mouse adipocytes. Diabetes 2011;60:1519–1527
34. Qiao L, Zou C, van der Westhuyzen DR, Shao J. Adiponectin reduces plasma triglyceride by increasing VLDL triglyceride catabolism. Diabetes 2008;57:1824–1833
35. Qiao L, Maclean PS, Schaack J, et al. C/EBPalpha regulates human adiponectin gene transcription through an intronic enhancer. Diabetes 2005;54:1744–1754
36. He L, Chinnery PF, Durham SE, et al. Detection and quantification of mitochondrial DNA deletions in individual cells by real-time PCR. Nucleic Acids Res 2002;30:e68
37. Hallberg M, Morganstein DL, Kiskinis E, et al. A functional interaction between RIP140 and PGC-1alpha regulates the expression of the lipid droplet protein CIDEA. Mol Cell Biol 2008;28:6785–6795
38. Boppart MD, Hirshman MF, Sakamoto K, Fielding RA, Goodyear LJ. Static stretch increases c-Jun NH2-terminal kinase activity and p38 phosphorylation in rat skeletal muscle. Am J Physiol Cell Physiol 2001;280:C352–C358
39. Li X, Monks B, Ge Q, Birnbaum MJ. Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. Nature 2007;447:1012–1016
40. Wu JJ, Roth RJ, Anderson EJ, et al. Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. Cell Metab 2006;4:61–73