Adiponectin Inhibits Cell Proliferation by Interacting with Several Growth Factors in an Oligomerization-dependent Manner

Yu Wang†‡§§, Karen S. L. Lam**, Jian Yu Xu**, Gang Lu†, Lance Yi Xu¶, Garth J. S. Cooper¶, and Aimin Xu**

From the †Genome Research Centre and the Departments of §Biochemistry and **Medicine, the University of Hong Kong, Hong Kong, China and the ¶Center for Molecular Biodiscovery and School of Biological Sciences, University of Auckland, Auckland 1001, New Zealand

This work was supported by grants from the Marsden funds of the Royal Society of New Zealand (to Y. W.), Hong Kong Research Council Grant HKU 7486/04M (to A. X.), and a research and conference grant to the University of Hong Kong (to K. S. L. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received for publication, February 1, 2005
Published, JBC Papers in Press, February 25, 2005, DOI 10.1074/jbc.M501149200

© 2005 by The American Society for Biochemistry and Molecular Biology, Inc.

Adiponectin, an adipocyte-specific secretory protein, is present in serum as three oligomeric complexes. Apart from its roles as an anti-diabetic and anti-atherogenic hormone, adiponectin has been implicated as an important regulator of cell growth and tissue remodeling. Here we show that some of these functions might be mediated by the specific interactions of adiponectin with several important growth factors. Among six different growth factors examined, adiponectin was found to bind with platelet-derived growth factor BB (PDGF-BB), basic fibroblast growth factor (FGF), and heparin-binding epidermal growth factor-like growth factor (HB EGF) with distinct affinities. The bindings of adiponectin with these growth factors are oligomerization-dependent. PDGF-BB bound to the high molecular weight (HMW) and middle molecular weight (MMW) complexes, but not to the low molecular weight (LMW) complex of adiponectin. Basic FGF preferentially interacted with the HMW form, whereas HB EGF bound to all three forms with comparable affinities. These three growth factors did not compete with each other for their bindings to adiponectin, suggesting the involvement of distinct binding sites. The interactions of adiponectin with PDGF-BB, basic FGF, and HB EGF precluded the bindings to their respective membrane receptors and attenuated the DNA synthesis and cell proliferation induced by these growth factors. Small interfering RNA-mediated down-regulation of adiponectin receptors did not affect the suppressive effects of adiponectin on cell proliferation stimulated by these growth factors. These data collectively suggest that the oligomeric complexes of adiponectin can modulate the biological actions of several growth factors by controlling their bioavailability at a pre-receptor level and that this effect might partly account for the anti-atherogenic, anti-angiogenic, and anti-proliferative functions of adiponectin.

Adiponectin (also called AdipoQ, ACRP30, GBP28, and aPM1) is an abundant circulating hormone predominantly expressed from adipose tissue (1–4). The protein belongs to the complement factor C1q-like superfamily and is composed of an NH2-terminal collagenous region (with a 22-G12-galactosyl group) and a COOH-terminal globular domain. We have previously demonstrated that several conserved lysine residues within the collagenous domain of adiponectin are hydroxylated and glycosylated by the addition of a glucosyl-α,1,2-galactosyl group (5, 6). In the circulation, adiponectin forms different oligomeric complexes, including high molecular weight (HMW), middle molecular weight (MMW), and low molecular weight (LMW) species (5, 7–9). Growing evidence suggests that adiponectin is an insulin-sensitizing hormone with direct anti-diabetic, anti-atherogenic, and anti-inflammatory activities. The circulating levels of adiponectin are decreased in obese individuals and patients with type 2 diabetes, hypertension, and coronary heart diseases (10–13). Adiponectin replacement therapy has been shown to decrease hyperglycemia and hypertriglyceridemia, restore insulin sensitivity, and alleviate fatty liver diseases in mice (14–17). The direct anti-atherogenic role of adiponectin was confirmed in vivo by two more recent studies, which demonstrated that atherosclerosis in apoE-deficient mice is markedly alleviated by either the injection of adenoviruses that express adiponectin (18) or by crossing transgenic mice expressing the globular domain of adiponectin with apoE-deficient mice (19). On the other hand, depletion of the adiponectin gene in mice caused much more neointimal formation in response to external vascular cuff injury than that of wild-type mice (20, 21). Adiponectin has been shown to accumulate in injured blood vessel walls and dose-dependently inhibits tumor necrosis factor α-induced cell adhesion in human aortic endothelial cells (22). It can inhibit the proliferation of myelomonocytic progenitors and phagocytic activity by macrophages and also suppress foam cell formation by decreasing low density lipoprotein uptake (23).

Growing evidence suggests that adiponectin is also an important regulator of cell proliferation. Recombinant adiponectin could directly inhibit pathological angiogenesis and suppress tumor growth in mice (24). In cultured smooth muscle cells, adiponectin attenuated DNA synthesis, cell proliferation, and migration induced by several growth factors (25). The mechanism that underlies the anti-proliferative effects of adi-
Adiponectin is still poorly understood. In the present study, we have used both a solid phase binding assay and surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry to investigate the interactions of adiponectin with several common growth factors involved in cell proliferation. We found that adiponectin selectively binds with platelet-derived growth factor BB (PDGF-BB), heparin-binding epidermal growth factor-like growth factor (HB EGF), and basic fibroblast growth factor (basic FGF) and inhibits the interactions of these growth factors with their membrane receptors. We further analyzed the molecular and structural basis that underlies these interactions and found that the binding of adiponectin with these growth factors involves different oligomeric complexes and distinct binding sites.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human aortic smooth muscle cells (HASMCs) and 3T3-L1 cells were purchased from American Type Culture Collection (Manassas, VA). Recombinant human PDGF-BB, PDGF-AA, HB EGF, basic fibroblast growth factor (bFGF), and endothelial cell growth factor (EGF) were purchased from R&D Systems (Minneapolis, MN). [methyl-3H]thymidine, [125I]PDGF-BB, [125I]basic FGF, and [125I]EGF were from GE Healthcare (Chalfont St. Giles, United Kingdom). 125I-IGF-I was from PerkinElmer Life Sciences. Recombinant PDGF-AA and HB EGF were labeled by a [125I]-labeled Bolton and Hunter reagent according to the manufacturer's instructions (GE Healthcare). PS20 ProteinChip® array was from Ciphergen Biosystems Inc. (Fremont, California). The anti-FLAG M2 monoclonal antibody affinity gel was from Sigma. Lipofectamine 2000 and the cell culture media were purchased from Invitrogen.

**Expression and Purification of Recombinant Murine Adiponectin and Its Variant**—The wild-type murine adiponectin with a FLAG epitope tagged at the COOH terminus was expressed in HEK293 cells and purified using the anti-FLAG M2 monoclonal antibody affinity gel from the conditioned culture medium as we described previously (6). The mammalian expression vector pcDNA-Ad-F (6) was used as a template, and the mutagenic oligonucleotide primers designed with a codon change from TGT to GCT for the residue cysteine 39 replaced by alanine, was generated using the QuickChange site-directed mutagenesis kit (Stratagene). Briefly, the wild-type adiponectin expression vector pcDNA-Ad-F (6) was used as a template, and the mutagenic oligonucleotide primers designed with a codon change from TGT to GCT (6) were used for amplification to generate the construct. The mutation was confirmed by DNA sequencing. This construct was transfected into HEK293 cells, which were then subjected to selection using 1 mg/ml G418 to generate a cell line that stably expresses the adiponectin variant C39A. The mutant protein was purified from the conditioned culture medium as described above. The purity was >95% as determined by SDS-PAGE.

**Gel Filtration Chromatography**—The purified recombinant murine adiponectin diluted with PBS to 1 ml and then separated on a Hi-Load 16/60 Superdex™ 200 prep grade column using the BioCAD™ work station perfusion chromatography system (PerSeptive Biosystems) according to the manufacturer's instructions. The fractions containing different oligomeric forms of adiponectin were collected, pooled, concentrated, and stored at −80 °C until use.

**Binding of [125I]-Labeled Growth Factors to Adiponectin**—100 μl of total or different forms of adiponectin in PBS (5 μg/ml) was coated onto the 96-well polystyrene plates overnight at 4 °C. The wells were rinsed with washing buffer (PBS containing 0.05% Tween 20) blocked with 200 μl of 2% BSA (w/v) in PBS for 90 min at room temperature, and then washed three times with the washing buffer. [125I]-labeled growth factors (PDGF-AA, PDGF-BB, basic FGF, EGF, HB EGF, and IGF-I) were added to wells at different concentrations and incubated for 90 min at room temperature on an orbital shaker. For the competition experiment, adiponectin was added into the well and incubated for the last 3 h with 1N NaOH, and the total binding was determined by measuring the radioactivity using a γ-counter (NuclearChicago, IL). The binding was determined by the addition of 500-fold excess of the respective unlabeled growth factors and subtracted from the total binding.

**Inhibition of Adiponectin Receptor (AdipoR) Expression in HASMCs**—The target-specific RNAi duplexes were designed based on the published sequences for human AdipoR1 and AdipoR2 (27). The sequences of the sense RNAi for AdipoR1 and AdipoR2 are GCUAAGGCAACACGC-UACUCUGGA and CCAAGGCAAGGATUCA (27). Nonspecific BLAST search analysis against the human genome sequence revealed that the sequences of both fragments are specific to their respective genes. The oligonucleotide 5′-AACGTACCGGAAATTCTGGA-3′, targeting the coding region of the firefly luciferase gene, was used as the RNAi control. All of the RNAi duplexes were generated by Invitrogen. 500 pmol RNAi duplexes were transfected into HASMCs using Lipofectamine 2000 (Invitrogen). At different time intervals after transfection, the mRNA abundance of the human AdipoR1 and AdipoR2 was quantified by quantitative real time PCR as described previously (27).

**RESULTS**

**Adiponectin Can Bind Specifically with PDGF-BB, HB EGF, and Basic FGF**—Adiponectin is a glycoprotein that forms multimeric complexes in the circulation (5, 7–9). Many circulating glycoprotein complexes, such as α2-macroglobulin and thrombomodulin 1, modulate cell proliferation by interaction with growth factors (28–30). We therefore investigated the interactions of adiponectin with a panel of well characterized growth factors involved in cell proliferation. To this end, recombinant murine adiponectin immobilized in a polystyrene well was used as a probe to detect its interactions with the liquid phase [125I]-labeled growth factors, including PDGF-BB, PDGF-AA, basic FGF, EGF, IGF-I, and HB EGF. The binding between [125I]-labeled growth factors and immobilized mouse adiponectin was measured in a buffer with physiological ionic strength. The interaction between the labeled growth factors and immobi-
lized BSA was also measured in parallel and subtracted from the total binding to obtain the specific bindings in each concentration point. This analysis revealed that adiponectin selectively bound with increasing concentrations of $^{125}$I-labeled PDGF-BB, HB EGF, and basic FGF, but not PDGF-AA, IGF-I, and EGF (Fig. 1). The bindings of immobilized adiponectin with $^{125}$I-PDGF-BB, HB EGF, and basic FGF were substantially decreased following the addition of excessive amounts of recombinant adiponectin into the liquid phase, suggesting that the bindings are reversible. The dissociation constants for PDGF-BB, HB EGF, and basic FGF were 23.22 pM, 167.2 pM, and 148.4 pM, respectively. This result suggests that PDGF-BB has the highest binding affinity with adiponectin, followed by HB EGF and basic FGF. The specificity of the interactions between adiponectin and the three growth factors was further confirmed by SELDI-TOF analysis, which showed that PDGF-BB, basic FGF, and HB EGF selectively bound to a “biochip” coated with adiponectin, but not to BSA or mouse IgG (Fig. 2). Taken together, these data suggest that adiponectin can reversibly and specifically bind to PDGF-BB, HB EGF, and basic FGF with different affinities.

**PDGF-BB, Basic FGF, and HB EGF Interact with Different Oligomeric Complexes of Adiponectin—**Circulating adiponectin is present predominantly as three major oligomeric complexes, and each of them might possess distinct functions (7, 8). To determine the role of adiponectin oligomerization in its binding with the three growth factors, we isolated different oligomeric complexes of adiponectin. Both gel filtration chromatography and non-reducing, non-heating SDS-PAGE analysis revealed that recombinant adiponectin expressed from HEK293 cells formed oligomeric complexes of the HMW, MMW, and LMW species (Fig. 3, A and B), a pattern similar to that of endogenous adiponectin as reported by others (7, 8). The three oligomeric forms of adiponectin were then immobilized individually on a polystyrene plate to study their interactions with PDGF-BB, basic FGF, and HB EGF (Fig. 3, C–E). This analysis revealed that the three growth factors bound to different oligomeric complexes of adiponectin with distinct affinities. PDGF-BB interacted with both HMW and MMW adiponectin with a similar affinity, whereas it showed no binding with the LMW form. Basic FGF bound selectively to the HMW form but not to the MMW and LMW forms. On the other hand, HB EGF interacted with all of the three oligomeric complexes of adiponectin with a comparable affinity. In addition, these three growth factors did not compete with each other for their respective bindings to adiponectin (data not shown). These results collectively suggest that the binding of adiponectin to the three growth factors involves its different oligomeric complexes and distinct interaction sites.

To further confirm the above findings, we generated a recombinant adiponectin variant in which cysteine 39, at its collagenous domain, is replaced by alanine (C39A). This cysteine residue has previously been shown to be essential for the formation of MMW and HMW complexes of adiponectin (7, 9). In line with these previous reports, our results demonstrated that the adiponectin variant (C39A) could only form the LMW form but not the other two high order complexes (Fig. 4). Binding analysis revealed that this form of adiponectin could interact only with HB EGF but not with PDGF-BB and basic FGF.

**Adiponectin Inhibits the Binding of $^{125}$I-Labeled PDGF-BB, Basic FGF, and HB EGF to Their Receptors in HASMCs and 3T3-L1 Cells—**The results described above suggest that adiponectin might act as a carrier protein for PDGF-BB, basic FGF, and HB EGF to regulate the biological activities of these growth factors. We next investigated the effects of adiponectin on the receptor-binding activities of $^{125}$I-labeled PDGF-BB, basic FGF, and HB EGF on HASMCs and 3T3-L1 cells, both of which contain the functional receptors for these three growth factors. Adiponectin inhibited the bindings of $^{125}$I-labeled PDGF-BB, basic FGF, and HB EGF to their respective receptors in both HASMCs (Fig. 5A) and 3T3-L1 cells (data not shown) in a dose-dependent manner. The potency of the inhibition was highest for PDGF-BB, followed by HB EGF and basic FGF. The adiponectin variant (C39A) had little or no inhibitory effect on the receptor binding of PDGF-BB and basic FGF in HASMCs. On the other hand, the mutant protein could still attenuate the binding of HB EGF to its receptor (Fig. 5B).

**Adiponectin Attenuates DNA Synthesis and Cell Proliferation Stimulated by PDGF-BB, Basic FGF, and HB EGF—**We next investigated whether the binding of adiponectin with the three growth factors eventually leads to the attenuation of their mitogenic activities. Growth-arrested HASMCs were stimulated with different mitogens and/or adiponectin, and DNA synthesis was subsequently measured using a $^{3}$Hthyidine incorporation assay. As shown in Fig. 6A, 10 ng/ml PDGF-BB, basic FGF, and HB EGF increased DNA synthesis by...
3.1-, 2.2-, and 1.9-fold over the basal level, respectively. Treatment with 5 g/ml wild-type adiponectin attenuated the 
[3H]thymidine incorporation induced by PDGF-BB, basic FGF, and HB EGF by 83, 71, and 59%, respectively, whereas it had 
no suppressive effect on basal and phorbol 12-myristate 13-
acetate-induced DNA synthesis (data not shown). The inhibi-
tory effects of adiponectin on cell proliferation induced by the 
three growth factors were confirmed by cell counting. PDGF-
BB, basic FGF, and HB EGF-mediated increases of cell num-
bers were markedly inhibited by 5 g/ml of adiponectin (Fig. 
6B). In line with the binding data, the adiponectin variant
(C39A) decreased DNA synthesis and cell proliferation induced 
by HB EGF significantly, but exerted little effect on the DNA 
synthesis and cell proliferation induced by PDGF-BB and basic 
FGF. A similar effect of adiponectin on the DNA synthesis and 
cell proliferation induced by the three growth factors was also 
observed in 3T3-L1 fibroblast cells (data not shown).

Adiponectin Receptor 1 (AdipoR1) and Adiponectin Receptor 2 
(AdipoR2) Are Not Involved in Adiponectin-mediated Suppres-
sion of Cell Proliferation Induced by PDGF-BB, Basic FGF, and 
HB EGF—AdipoR1 and AdipoR2 are the two recently cloned 
adiponectin receptors that are proposed to mediate the multi-

FIG. 2. SELDI-TOF analysis of the interactions between adiponectin and PDGF BB, basic FGF, and HB EGF. The PS20 ProteinChip® 
array was coated with 5 μg of BSA (A, C, and E) or murine adiponectin (B, D, F, and G) as described under “Experimental Procedures.” The array 
was then incubated without (G) or with 50 ng of PDGF-BB (A and B), basic FGF (C and D), HB EGF (E and F) at room temperature for 1 h. After 
washing with PBS and water, the bound growth factors were detected using the SELDI-TOF machine. Note that all three growth factors bind 
specifically to adiponectin but not to BSA or IgG (data not shown).

FIG. 3. PDGF-BB, basic FGF, and 
HB EGF bind to distinct oligomeric 
complexes of adiponectin. A, separa-
tion of different oligomeric forms of re-
combinant murine adiponectin by gel fil-
tration chromatography. B, SDS-PAGE 
analysis of adiponectin. Total adiponectin 
(lane I) and adiponectin from well re-
solved fractions of peak 1 (lane II), peak 2 
(lane III), and peak 3 (lane IV) in panel A 
were separated on a 4–20% gradient gel 
under non-heating and non-reducing con-
ditions and visualized by Coomassie Bril-
liant Blue R250 staining. 5 μg/ml HWM, 
MMW, and LMW forms of adiponectin 
were immobilized onto a polystyrene 
plate separately, and the binding of 
PDGF-BB (C), basic FGF (D), and HB 
EGF (E) to different oligomeric forms of 
adiponectin were measured as in Fig. 1.
ple biological functions of adiponectin in many cell lines (27). Indeed, our PCR analysis revealed that both receptors were expressed in HASMCs (data not shown). We next used small interfering RNA to specifically down-regulate the expressions of AdipoR1 and AdipoR2. 24 h after transfection with their respective RNAi duplexes, the expressions of AdipoR1 and AdipoR2 were decreased by 79 and 76%, respectively (Fig. 7A). Notably, the marked down-regulation of both adiponectin receptors in HASMCs did not attenuate the suppressive effects of adiponectin on DNA synthesis induced by PDGF-BB, basic FGF, and HB EGF (Fig. 7A), suggesting that neither AdipoR1 nor AdipoR2 is required for the anti-proliferative effect of adiponectin on this cell line.

**DISCUSSION**

In addition to its function as a metabolic hormone, growing evidence suggest that adiponectin plays a regulatory role in cell growth, angiogenesis, and tissue remodeling. Adiponectin has been shown to inhibit proliferation of aortic smooth muscle cells, myelomonocytic cells, endothelial cells, and hepatic stellate cells (24, 25, 32, 33). In this study, we provide evidence that adiponectin selectively binds with several mitogenic growth factors that can induce cell proliferation in many types of cells. The interaction of adiponectin with these growth factors can preclude their binding to the membrane receptors and lead to the attenuation of their mitogenic actions, suggesting that the anti-proliferative effect of adiponectin is at least partly due to its selective sequestration of growth factors at a pre-receptor level. This conclusion was also supported by the fact that adiponectin alone has no effect on cell proliferation (Fig. 6) and that the suppressive effect of adiponectin on growth factor-induced cell proliferation does not rely on its functional receptors (Fig. 7).

In line with our results, a recent report by Arita et al. (25) also demonstrated the binding of adiponectin with PDGF-BB. However, their study failed to detect the interaction between adiponectin and HB EGF. The discrepancy between our study and that of Arita et al. might be due to the different sources of recombinant adiponectin used for the assays. The recombinant adiponectin used in the present study was generated from mammalian cells and posttranslationally modified by hydroxylation and glycosylation (6). On the other hand, the adiponectin used by Arita et al. was produced from prokaryotic cells, which lacked posttranslational modifications. It is possible that posttranslational modifications of adiponectin might be involved in the binding of this protein with HB EGF. In line with this speculation, a recent study from Lodish and co-workers found that only the posttranslationally modified adiponectin generated from mammalian cells, but not adiponectin from *Escherichia coli*, could bind with its potential receptor T-cadherin (34).

Another novel finding of the present study is that the binding of adiponectin with different growth factors involves different oligomeric forms and distinct binding sites. The three growth factors examined in this study have distinct binding affinities and do not compete with each other for their respective binding sites to adiponectin. PDGF-BB binds with both the HMW and MMW forms of adiponectin but not with the LMW form. Basic FGF binds preferentially with HMW adiponectin, whereas HB EGF interacts with all of the three oligomeric complexes with comparable affinity. These data collectively suggest that the high order structures of adiponectin, instead of a binding motif/sequence, are critical for its interaction with different growth factors. In line with our findings, several recent reports demonstrated that oligomerization plays an essential role in determining many biological activities of adiponectin. Different oligomeric complexes of adiponectin have been shown to activate different pathways in their target tissues. Tsao et al. (7) dem-
onstrated that only the LMW form of adiponectin, but not the HMW or MMW form, stimulated AMP-activated protein kinase pathways in the muscle tissue. On the other hand, Pajvani et al. (35) showed that the HMW complex was the most active form of adiponectin in depressing blood glucose levels in mice. Our finding further supports the notion that different oligomeric complexes of adiponectin possess distinct functions and highlights the importance for a further understanding of the molecular events involved in the regulation of adiponectin oligomeric complex assembly.

The role of adiponectin as a binding protein for multiple growth factors is reminiscent of 2-macroglobulin and thrombospondin 1, both of which are highly abundant circulating glycoproteins and form large oligomeric complexes. 2-Macroglobulin has been shown to bind with a variety of growth factors and cytokines, including PDGF-BB, basic FGF, tumor necrosis factor-α, transforming growth factor-β, vascular endothelial growth factor, etc. (29, 36–38). The interactions can either enhance or suppress the biological activities of these growth factors in a conformation-dependent manner. Thrombospondin 1, a multidomain glycoprotein involved in cell-matrix interactions, has been shown to interact with PDGF-BB and assist in the targeting of this growth factor to its receptors on vascular smooth muscle cells (30, 39). Interestingly, previous studies have shown that endogenous adiponectin is physically associated with the α2-macroglobulin and tightly bound with other extracellular matrix proteins (40, 41). We have also found that adiponectin interacts with thrombospondin-1.

Based on these data, it is tempting to propose that adiponectin, together with the α2-macroglobulin and thrombospondin-1, might form a complex network at the extracellular matrix and coordinately control the cell growth and tissue remodeling by regulating the local concentration and bio-availability of different growth factors.

It is of interest to note that all three adiponectin binding growth factors identified in this study are potent mitogens of vascular smooth muscle cells and are strongly implicated in the pathogenesis of atherosclerosis (42). The hallmark of this disease is uncontrolled migration and proliferation of smooth muscle cells, which results in thickening of the vascular wall and leads to the eventual occlusion of the arteries. PDGF-BB, basic FGF, and HB EGF can be made by most cells in atherosclerotic lesions and are generally considered to be the major factors responsible for the excessive proliferation of smooth muscle cells. Adiponectin has been shown to accumulate in the sub-endothelial space of injured vessel walls of human subjects (40). These data collectively suggest that sequestration of locally produced atherogenic growth factors by adiponectin might contribute to its protective effect on vascular injury and atherosclerosis.

In addition to their roles in smooth muscle proliferation, PDGF-BB, basic FGF, and HB EGF are well known to be the important regulators of angiogenesis, carcinogenesis, morphogenesis, chemostasis, and inflammation (43–45). Adiponectin might also regulate these pathophysiological processes by reg-

---

2 Y. Wang, K. S. L. Lam, J. Y. Xu, G. Lu, L. Y. Xu, G. J. S. Cooper, and A. Xu, unpublished data.
ulating the bioavailability of the three growth factors. Indeed, several recent studies have demonstrated the anti-cardiogenic and anti-angiogenic activities of adiponectin in animal models (24, 46). Further understanding might arise from the structural basis that underlies these interactions which might help in the development of novel therapeutic strategies for atherosclerosis and cancer.

Acknowledgment—We thank Dr. Ian Melhado for correcting the manuscript.

REFERENCES

1. Hu, E., Liang, P., and Spiegelman, B. M. (1996) J. Biol. Chem. 271, 10697–10703
2. Maeda, K., Okubo, K., Shimomura, I., Funahashi, T., Matsuzawa, Y., and Matsuura, K. (1996) Biochem. Biophys. Res. Commun. 221, 286–289
3. Nakano, Y., Tobe, T., Cho-Miura, N. H., Maeda, K., and Tomita, M. (1996) J. Biochem. (Tokyo) 120, 803–812
4. Scherer, P. E., Williams, S., Fogliano, M., Baldini, G., and Lodish, H. F. (1995) Biochem. Biophys. Res. Commun. 210, 698–703
5. Wang, Y., Lu, G., Wong, W. P., Vliegenthart, J. F., Lam, K. S., Gerwig, G. J., and Cooper, G. J. (2002) J. Biol. Chem. 277, 40352–40363
6. Wang, Y., Xu, A., Knight, C., Xu, L. Y., and Cooper, G. J. (2004) J. Biol. Chem. 279, 15211–15213
7. Tsao, T. S., Murrey, H. K., Uchida, S., Takakuwa, K., Matsui, J., Takata, M., Eto, K., Terauchi, Y., Uchida, S., Kiso, S., Murakami, K., Ohteki, T., Uchida, S., Takekawa, S., Waki, H., Tsuno, N. H., Shibata, Y., Terauchi, Y., Froguel, P., Tobe, K., Koyasu, S., Ito, Y., Tomita, T., Shiramizu, T., Shimizu, T., Nishida, M., and Takahashi, M. (2000) J Biol. Chem. 275, 3933–3942
8. Waki, H., Yamauchi, T., Kamada, Y., Terauchi, Y., Yamauchi, T., Kamada, Y., Terauchi, Y., and Tataranni, P. A. (2001) Arterioscler. Thromb. Vasc. Biol. 21, 1730–1737
9. Pajvani, U. B., Hawkins, M., Combs, T. P., Rajala, M. W., Doebber, T., Berger, A. H., and Scherer, P. E. (2001) J. Cell. Biol. 153, 85–89
10. Arita, Y., Kihara, S., Ouchi, N., Maeda, K., Miyagishi, M., Shudo, K., Kuriyama, H., Nishida, M., Yamauchi, T., Akaboshi, Y., Funahashi, T., and Matsuzawa, Y. (2002) J. Biol. Chem. 276, 27890–27895
11. Weyer, C., Funahashi, T., Matsuzawa, Y., and Matsuzawa, Y. (2003) Biochem. Biophys. Res. Commun. 304, 450–453
