Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 333 (2005) 79–87
BBRC
www.elsevier.com/locate/ybbrc

Adiponectin activates c-Jun NH_2-terminal kinase and inhibits signal transducer and activator of transcription 3 ☆

Toshiaki Miyazaki, Jeffrey D. Bub, Miwa Uzuki, Yoshiki Iwamoto *

Department of Urology, Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI 53226-0509, USA

Received 13 May 2005
Available online 26 May 2005

Abstract

Adiponectin, a major adipose cytokine, plays a crucial role in the inhibition of metabolic syndrome by acting on such cell types as muscle cells and hepatocytes. Furthermore, evidence suggests that adiponectin may influence cancer pathogenesis. Adiponectin occurs in non-proteolytic (full-length adiponectin: f-adiponectin) and proteolytic (globular adiponectin: g-adiponectin) forms in various oligomeric states. Different forms of adiponectin show distinct biological effects through differential activation of downstream signaling pathways. Here we identify c-Jun NH_2-terminal kinase (JNK), and signal transducer and activator of transcription 3 (STAT3) as common downstream effectors of f- and g-adiponectin. f- and g-adiponectin both stimulate JNK activation in prostate cancer DU145, PC-3, and LNCaP-FGC cells, hepatocellular carcinoma HepG2 cells, and C2C12 myoblasts. Furthermore, both f- and g-adiponectin drastically suppress constitutive STAT3 activation in DU145 and HepG2 cells. These suggest that JNK and STAT3 may constitute a universal signaling pathway to mediate adiponectin's pathophysiological effects on metabolic syndrome and cancer.

Keywords: Adiponectin; c-Jun NH_2-terminal kinase; Signal transducer and activator of transcription 3; Metabolic syndrome; Obesity; Prostate cancer; Hepatocellular carcinoma

Adiponectin is a cytokine encoded by a gene that is expressed most abundantly and highly specifically in adipose tissue [1–3]. Plasma levels of adiponectin correlate negatively with body mass index [4] and visceral fat accumulation [5]. Furthermore, low levels of plasma adiponectin are associated with such obesity-related disorders as diabetes mellitus [6], coronary artery (atherosclerotic) disease [7], and hypertension [8]. Mouse model studies have demonstrated crucial roles of adiponectin in pathogenic alterations of these disorders [9–12]. In addition, several lines of evidence indicate that adiponectin may influence cancer pathogenesis. Circulating adiponectin levels are inversely associated with an increased risk of breast [13,14] and endometrial [15,16] cancer, and breast tumors arising in women with low adiponectin levels are more likely to show a biologically aggressive phenotype [14]. Furthermore, adiponectin has been shown to inhibit cell proliferation and induce apoptosis in leukemia cells [17] and to suppress tumor growth in mice, most likely due to inhibition of neovascularization through suppression of endothelial cell proliferation, migration, and survival [18].

Adiponectin is found in both non-proteolytic (full-length adiponectin: f-adiponectin) and proteolytic forms. f-Adiponectin, a 30-kDa polypeptide, is comprised of an amino-terminal signal sequence, a variable domain, a collagen-like domain, and a carboxyl-terminal globular domain [1–3,19], and circulates at high levels in the bloodstream [4]. f-Adiponectin forms oligomers through disulfide bond formation mediated

☆ This study was supported in part by Department of Defense Grant DAMD17-02-1-0161 and by a grant from the Gustavus and Louise Pfeiffer Research Foundation.
* Corresponding author. Fax: +1 414 456 6516.
E-mail address: yiwamoto@mcw.edu (Y. Iwamoto).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.05.076

by Cys in the amino terminus and exists as three major oligomeric forms: a trimer, a hexamer, and a high molecular weight (HMW) form [20,21]. A proteolytic product of f-adiponectin containing the globular domain (globular adiponectin: g-adiponectin) exists as a trimer [22]. Differential biological functions have been reported among the oligomeric forms of f-adiponectin and g-adiponectin.

Adiponectin receptors, AdipoR1 and 2, have been identified in various tissues and cell types [23], although their expression in prostate has not previously been reported. AdipoR1 is abundantly expressed in skeletal muscle, while AdipoR2 is predominant in liver [23]. These receptors mediate cellular functions of both f- and g-adiponectin via activation of intracellular signaling pathways [23]. Several signaling molecules, such as 5′-AMP-activated protein kinase (AMPK), nuclear factor (NF)-κB, peroxisome proliferator-activated receptor (PPAR)-α, and p38 mitogen-activated protein (MAP) kinase, are known to mediate adiponectin-induced metabolic alterations. Some of the signaling pathways are differentially regulated by distinct forms of adiponectin. f-Adiponectin, but not g-adiponectin, down-regulates genes involved in gluconeogenesis through AMPK in liver [24]. In contrast, both f- and g-adiponectin stimulate fatty acid oxidation, glucose uptake, and lactate production via AMPK activation in C2C12 myocytes [24]. Interestingly, the trimeric form of f-adiponectin, but not the hexameric or HMW form, activates AMPK in C2C12 cells [25]. Activation of NF-κB by adiponectin in C2C12 cells is also oligomerization state-dependent: hexameric and HMW forms of f-adiponectin, but not trimeric f- or g-adiponectin, stimulate NF-κB activation in C2C12 cells [22,25]. Adiponectin regulates endothelial cell function as well. Contradictory to the observation that f-adiponectin inhibits angiogenesis through endothelial cell apoptosis in capillary endothelial cells in vitro and in a mouse tumor model in vivo [18], f-adiponectin stimulates angiogenesis via cross-talk between AMPK and Akt in umbilical vein endothelial cells (HUVECs) in vitro [26]. Furthermore, the HMW form of f-adiponectin selectively suppresses apoptosis with concomitant stimulation of AMPK in HUVECs [27]. These contradictions may be due to differences in endothelial cell types used here and in microenvironments between in vivo and in vitro [18]. f-Adiponectin also stimulates NO production mediated by Akt through AMPK in bovine aortic endothelial cells [28]. In addition, f-adiponectin inhibits tumor necrosis factor-α-induced NF-κB activation through protein kinase A in human aortic endothelial cells [29]. Although adiponectin has been demonstrated to modulate such signaling pathways, the effect of adiponectin on either c-Jun NH_2-terminal kinase (JNK) or signal transducer and activator of transcription (STAT) pathways has not been reported.

JNK constitutes one of the mammalian mitogen-activated protein (MAP) kinase families and is activated in response to various stimuli, including cytokines [30–33], and mediates the phosphorylation and activation of such transcription factors as c-Jun [34]. JNK is involved in the regulation of cell proliferation and apoptosis during various physiological and pathological events, including tumor development [35]. In addition, JNK plays a crucial role in obesity and insulin resistance [36,37].

The transcription factor STAT3 regulates diverse cellular functions, such as cell proliferation, survival, apoptosis, and differentiation [38]. In response to cytokines and growth factors, such as IL-6 family cytokines and leptin, STAT3 is activated through phosphorylation at Tyr-705 mediated by Janus kinase. The Tyr-phosphorylation allows STAT3 to dimerize, translocate to the nucleus, and activate transcription from target gene promoters containing a sis-inducible element (SIE) [39]. Constitutive STAT3 activation is crucial in malignant transformation and cancer progression [40]. Furthermore, STAT3 is involved in obesity and diabetes [41].

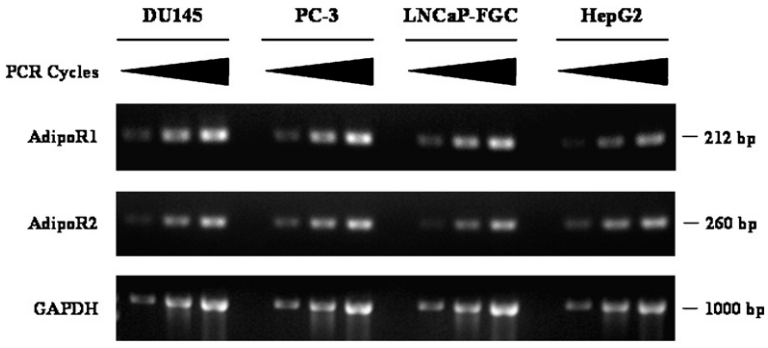


Fig. 1. Expression profiles of adiponectin receptor 1 and 2 in prostate cancer and hepatocellular carcinoma cells. Semi-quantitative reverse transcriptase-PCR analysis profiled the mRNA expression of adiponectin receptor isoforms in prostate cancer DU145, PC-3, and LNCaP-FGC cells, and hepatocellular carcinoma HepG2 cells. Adiponectin receptor has two isoforms (adiponectin receptor 1 and 2). After cells were deprived of serum for 24 h, total RNA was prepared and subjected to quantitative reverse transcriptase-PCR analysis. Amplifications were performed with 23, 26, and 29 cycles for adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) or 18, 21, and 24 cycles for GAPDH.

In this study, we determine the effects of f- and g-adiponectin on the activation of JNK and STAT3 in prostate cancer DU145, PC-3, and LNCaP-FGC cells, hepatocellular carcinoma HepG2 cells, and C2C12 myoblasts. We show that both f- and g-adiponectin stimulate JNK activation in these cell lines and inhibit STAT3 activation significantly in DU145 and HepG2 cells, in which STAT3 is constitutively activated. This suggests that JNK and STAT3 may be involved in the adiponectin regulation of metabolic disorders and that adiponectin may affect the pathogenesis of prostate cancer and hepatocellular carci-

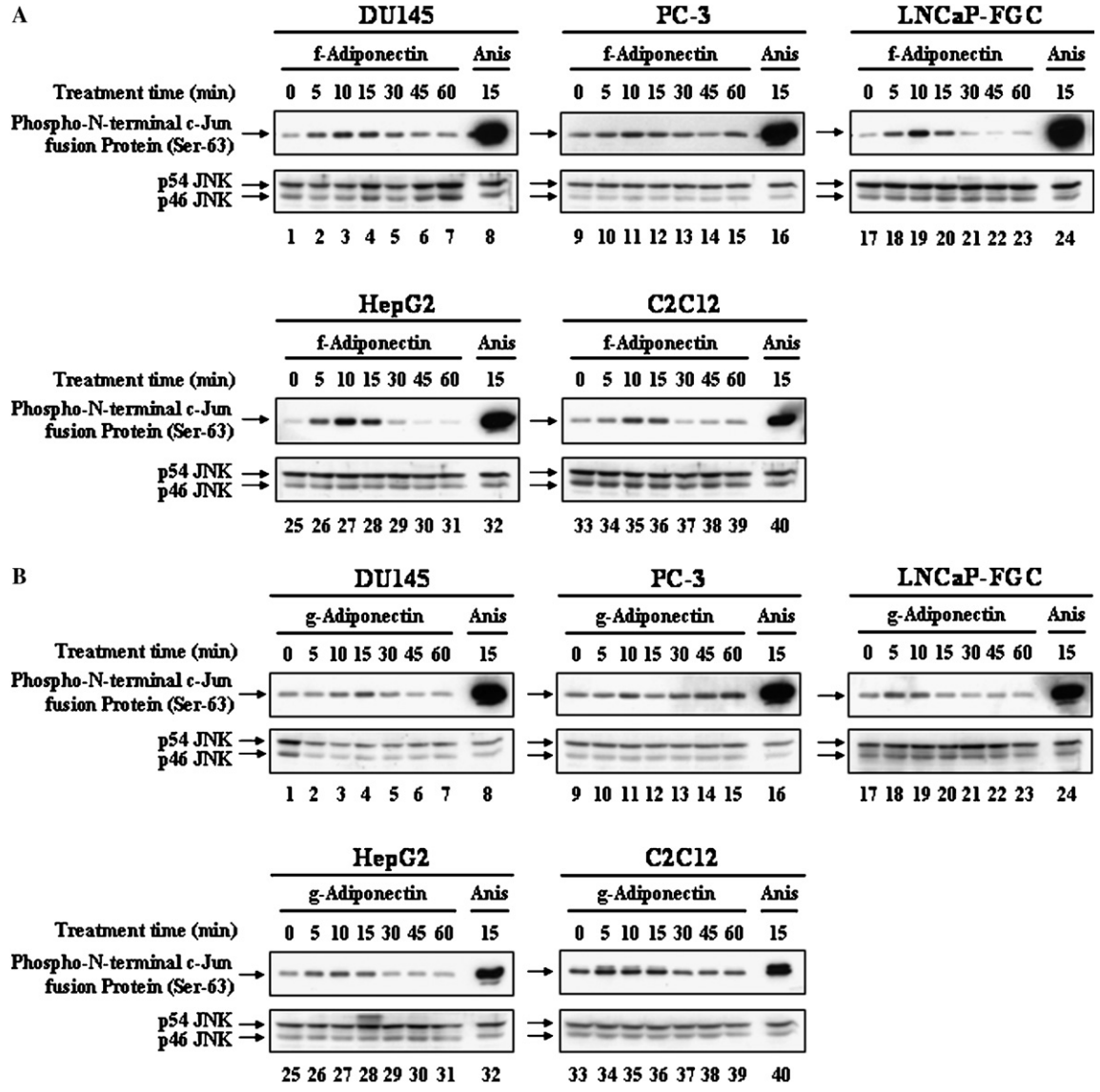


Fig. 2. (A) f-Adiponectin activates JNK in prostate cancer cells, hepatocellular carcinoma cells, and myoblasts. Prostate cancer DU145, PC-3, and LNCaP-FGC cells, hepatocellular carcinoma HepG2 cells, and C2C12 myoblasts were deprived of serum for 24 h and incubated in serum-free medium (lanes 1, 9, 17, 25, and 33) or the serum-free medium containing 1.0 μg/ml f-adiponectin (lanes 2–7, 10–15, 18–23, 26–31, and 34–39) or 10 μg/ml anisomycin (Anis) (lanes 8, 16, 24, 32, and 40) for indicated periods. Anisomycin served as a positive control to stimulate JNK activation. Cell lysates (250–500 μg protein) were subjected to the in vitro JNK assay with N-terminal c-Jun fusion protein as a substrate. Phosphorylation of the substrate protein on Ser-63 was detected by Western blot analysis using the specific antibody (phospho-N-terminal c-Jun fusion protein (Ser-63)). To normalize JNK activity to total JNK protein levels, cell lysates (100 μg protein) were applied to Western blot analysis using the anti-JNK antibody that detects both active and inactive forms of JNK (p54 JNK and p46 JNK). (B) g-Adiponectin activates JNK in prostate cancer cells, hepatocellular carcinoma cells, and myoblasts. Prostate cancer DU145, PC-3, and LNCaP-FGC cells, hepatocellular carcinoma HepG2 cells, and C2C12 myoblasts were deprived of serum for 24 h and incubated in serum-free medium (lanes 1, 9, 17, 25, and 33) or the serum-free medium containing 1.0 μg/ml g-adiponectin (lanes 2–7, 10–15, 18–23, 26-31, and 34–39) or 10 μg/ml anisomycin (*Anis*) (lanes 8, 16, 24, 32, and 40) for indicated periods. Anisomycin served as a positive control to stimulate JNK activation. Cell lysates (250–500 μg protein) were subjected to the in vitro JNK assay with N-terminal c-Jun fusion protein as a substrate. Phosphorylation of the substrate protein on Ser-63 was detected by Western blot analysis using the specific antibody (phospho-N-terminal c-Jun fusion protein (Ser-63)). To normalize JNK activity to total JNK protein levels, cell lysates (100 μg protein) were applied to Western blot analysis using the anti-JNK antibody that detects both active and inactive forms of JNK (p54 JNK and p46 JNK).

noma by acting on tumor cells directly through modulation of these molecules.

Materials and methods

Cytokines and antibodies. Recombinant human g-adiponectin was purchased from PeproTech (Rocky Hill, NJ). Recombinant human f-adiponectin was from R&D Systems (Minneapolis, MN). Velocity sedimentation with sucrose gradients revealed that the batch of human recombinant f-adiponectin used for this study was composed of the HMW and lower molecular weight forms with the majority of the HMW form (data not shown). The stress-activated protein kinase/JNK assay kit, anti-JNK, anti-c-Jun, anti-phospho-c-Jun (Ser-63), and anti-phospho-c-Jun (Ser-73) polyclonal antibodies were obtained from Cell Signaling Technology (Beverly, MA). The anti-STAT3 antibody for the supershift assay was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell lines and culture conditions. Human prostate cancer DU145, PC-3, and LNCaP-FGC cells, human hepatocellular carcinoma HepG2 cells, and murine immortalized C2C12 myoblasts were purchased from the American Type Culture Collection (Manassas, Virginia, USA). DU145, HepG2, and C2C12 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) plus penicillin (100 U/ml) and streptomycin (100 μg/ml). PC-3 and LNCaP-FGC cells were grown in RPMI 1640 supplemented with 10% FBS and antibiotics.

Semi-quantitative reverse transcriptase-PCR analysis. Total RNA was isolated using the TRIZOL standard technique from DU145, PC-3, LNCaP-FGC, and HepG2 cells deprived of serum for 24 h. cDNA was then generated from total RNA, and the reaction mixtures for PCR were prepared as described before [42]. PCR primers to detect AdipoR1 and AdipoR2 are as follows: AdipoR1 (forward: 5′-AG GACAACGACTATCTGCTAC-3′ and reverse: 5′-CATCCCAAAA ACCACCTTCTC-3′) and AdipoR2 (forward: 5′-AGAGAAAGTGG TGGGGAAAG and reverse: 5′-GGGCGAGGGAGGAAAATAAC-3′). PCR was carried out with an initial denaturing at 94 °C for 1 min, followed by 23–29 cycles consisting of denaturing at 94 °C for 1 min, annealing at 55 °C for 1 min, and extending at 72 °C for 1 min. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was employed as an internal standard to normalize sample variation as described previously [42]. PCR products were analyzed by electrophoresis on 2% agarose gels with ethidium bromide staining and compared after reaction cycles that showed DNA amplification in a linear range.

Cell lysate preparation. Seventy-percent confluent cells were deprived of serum for 24 h, followed by treatment with 1 μg/ml f- or g-adiponectin or 10 μg/ml anisomycin for indicated periods. After being rinsed with cold phosphate-buffered saline, cells were harvested using a scraper and collected by centrifugation at 700*g* for 10 min at 4 °C. The cell pellets were then homogenized in cell lysis buffer (20 mM Hepes, pH 7.9, 300 mM NaCl, 1 mM Na_3VO_4, 1 μg/ml leupeptin, 5 μg/ml aprotinin, 1 μg/ml pepstatin, and 1 mM phenylmethylsulfonyl fluoride) and rocked gently for 30 min at 4 °C. The homogenates were centrifuged at 15,000*g* for 5 min at 4 °C, and the supernatants were saved as cell lysates and stored at −80 °C until use.

In vitro JNK assay. Using the stress-activated protein kinase/JNK assay kit, JNK activity was assessed with a fusion protein of the c-Jun NH_2 terminus (amino acids 1–89) and glutathione *S*-transferase as a substrate according to the manufacturer's instructions with some modifications as reported before [42]. Briefly, JNK was precipitated in cell lysates (250–500 μg protein) with 2 μg glutathione–Sepharose beads immobilized by the fusion protein. The precipitates were rinsed with the cell lysis buffer three times, followed by incubation in 50 μl of the kinase buffer containing 100 μM ATP at 30 °C for 30 min. The reaction was terminated by adding the SDS sample buffer. The substrate fusion protein was separated by SDS–polyacrylamide gel electrophoresis, and its phosphorylation by JNK was determined by Western blot analysis using anti-phospho-c-Jun (Ser-63) polyclonal antibody. To normalize sample variation, JNK protein levels were also determined by Western blot analysis using anti-JNK antibody.

Measurement of c-Jun phosphorylation by Western blot analysis. Cell lysates (100 μg of protein) were electrophoresed on 10% SDS–polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After being washed and blocked, the membranes were hybridized with anti-phospho-c-Jun (Ser-63 or Ser-73) polyclonal antibody that specifically detects phosphorylated c-Jun molecules. The membranes were then stripped and re-hybridized with anti-c-Jun antibody that recognizes both phosphorylated and unphosphorylated c-Jun molecules to normalize sample variation.

Electromobility shift assay. Electromobility shift assay (EMSA) was performed as described previously [43]. Cell lysates containing 10 μg protein were subjected to the assay with ^{32}P-end-labeled, double-stranded oligonucleotide M67-SIE (forward: 5′-AATTC CATTTCC CGTAAATCCCTG-3′ and reverse: 5′-AATTCAGG GATTTACGG GAAATGG-3′) as a probe. The supershift assay was carried out to confirm the STAT3–DNA complex using anti-STAT3 antibody.

Results

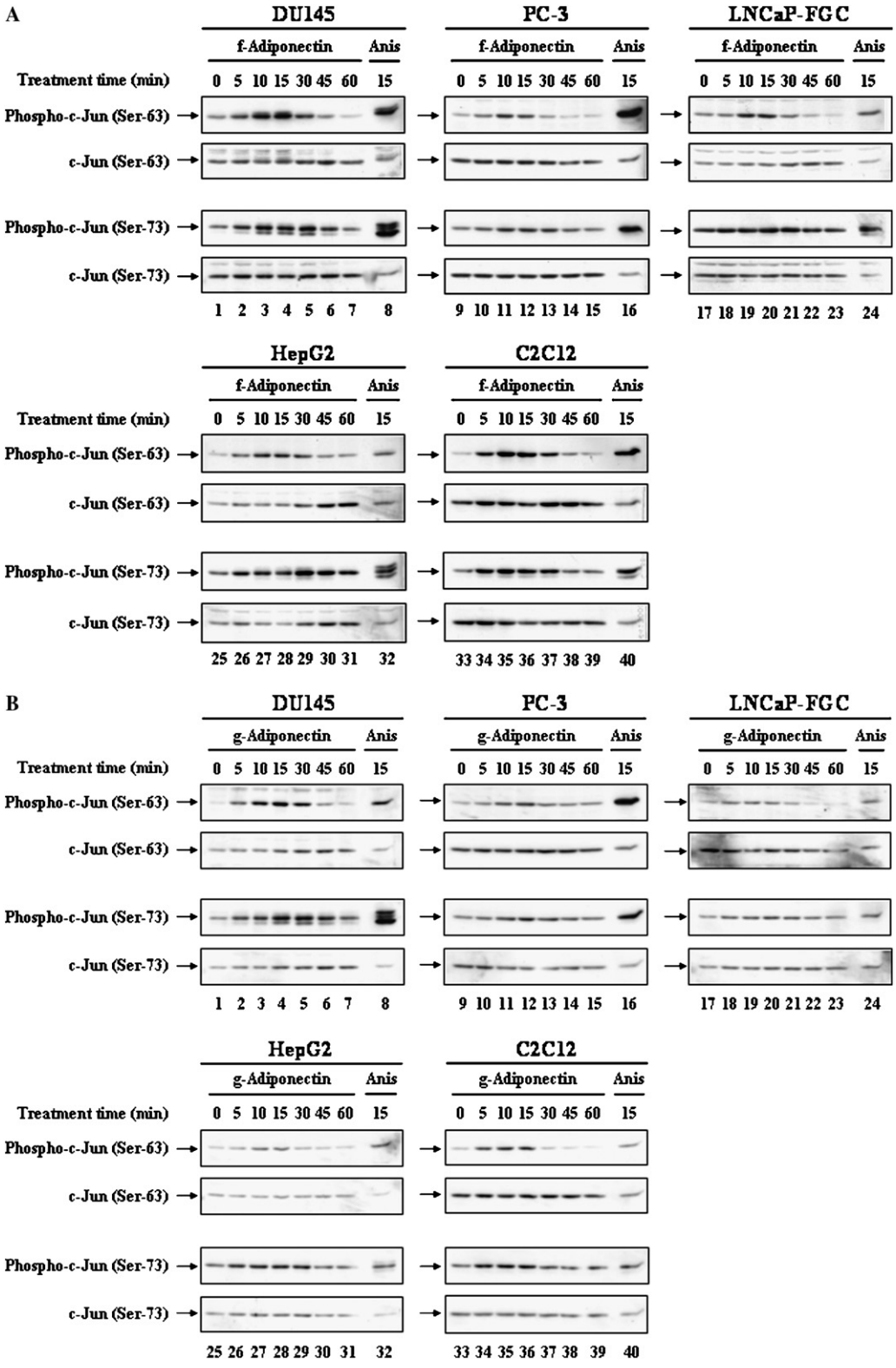
Expression of AdipoR1 and AdipoR2 by prostate cancer and hepatocellular carcinoma cells

The adiponectin receptors AdipoR1 and AdipoR2 have been identified in various tissues and cell types,

Fig. 3. (A) f-Adiponectin stimulates phosphorylation of c-Jun, an endogenous JNK substrate, in prostate cancer cells, hepatocellular carcinoma cells, and myoblasts. Prostate cancer DU145, PC-3, and LNCaP-FGC cells, hepatocellular carcinoma HepG2 cells, and C2C12 myoblasts were serum-starved for 24 h and treated with either 1.0 μg/ml f-adiponectin (lanes 2–7, 10–15, 18–23, 26–31, and 34–39) for indicated periods or 10 μg/ml anisomycin (Anis) (lanes 8, 16, 24, 32, and 40) for 15 min. Untreated (lanes 1, 9, 17, 25, and 33) and anisomycin-treated cells served as negative and positive controls. Cell lysates (100 μg protein) were subjected to Western blot analysis. c-Jun phosphorylation at Ser-63 and Ser-73 was determined with phospho-c-Jun (Ser-63) and (Ser-73) antibodies (phospho-c-Jun (Ser-63) and phospho-c-Jun (Ser-73)). To normalize c-Jun phosphorylation levels to total amounts of c-Jun protein, membranes probed with these antibodies were stripped and re-probed with the anti-c-Jun antibody that recognizes both phosphorylated and non-phosphorylated forms of c-Jun (c-Jun). (B) g-Adiponectin stimulates phosphorylation of c-Jun, an endogenous JNK substrate, in prostate cancer cells, hepatocellular carcinoma cells, and myoblasts. Prostate cancer DU145, PC-3, and LNCaP-FGC cells, hepatocellular carcinoma HepG2 cells, and C2C12 myoblasts were serum-starved for 24 h and treated with either 1.0 μg/ml g-adiponectin (lanes 2–7, 10–15, 18–23, 26–31, and 34–39) for indicated periods or 10 μg/ml anisomycin (Anis) (lanes 8, 16, 24, 32, and 40) for 15 min. Untreated (lanes 1, 9, 17, 25, and 33) and anisomycin-treated cells served as negative and positive controls. Cell lysates (100 μg protein) were subjected to Western blot analysis. c-Jun phosphorylation at Ser-63 and Ser-73 was determined with phospho-c-Jun (Ser-63) and (Ser-73) antibodies (phospho-c-Jun (Ser-63) and phospho-c-Jun (Ser-73)). To normalize c-Jun phosphorylation levels to total amounts of c-Jun protein, membranes probed with these antibodies were stripped and re-probed with the anti-c-Jun antibody that recognizes both phosphorylated and non-phosphorylated forms of c-Jun (c-Jun).

including myocytes and hepatocytes [23]. However, their expression by cancer cells has not been reported.

Using quantitative RT-PCR, we profiled expression of AdipoR1 and AdipoR2 in prostate cancer DU145, PC-3, and LNCaP-FGC cells, and hepatocellular carcinoma HepG2 cells (Fig. 1). All four cell lines expressed mRNA of both receptors. AdipoR1 and AdipoR2 were both expressed at similar levels in the three prostate cancer cell lines. HepG2 cells expressed more AdipoR2 and less AdipoR1 than prostate cancer cells. This is



consistent with a previous report that AdipoR2 is the predominant form expressed in liver [23].

Stimulation of JNK activation by f- and g-adiponectin

We examined JNK activity in prostate cancer DU145, PC-3, and LNCaP-FGC cells, hepatocellular carcinoma HepG2 cells, and C2C12 myoblasts treated with f- or g-adiponectin for periods up to 60 min. JNK was constitutively activated at low levels in all the five cell lines, and f-adiponectin further stimulated JNK activation, peaking at 10 min after the addition of f-adiponectin (Fig. 2A). Likewise, g-adiponectin stimulated JNK activation in all the cell lines (Fig. 2B). However, g-adiponectin maximized JNK activation at different time points varying between 5 and 15 min after cytokine addition among the cell lines.

Stimulation of c-Jun phosphorylation by f- and g-adiponectin

c-Jun is a physiological substrate for JNK [44]. JNK phosphorylates c-Jun at Ser-63 and Ser-73, and phosphorylation of these residues is required for c-Jun activation [44].

We examined c-Jun phosphorylation during f- or g-adiponectin treatment in DU145, PC-3, LNCaP-FGC, HepG2, and C2C12 cells. c-Jun was constitutively phosphorylated at Ser-63 and Ser-73 in all the five cell lines. f- and g-adiponectin both augmented c-Jun phosphorylation at Ser-63 in the five cell lines (Figs. 3A and B) in correlation with JNK activation (Figs. 2A and B). Both forms of adiponectin also stimulated Ser-73 phosphorylation in all the cell lines although the stimulation was quite modest in LNCaP-FGC cells (Figs. 3A and B). It should be noted that adiponectin induced Ser-63 phosphorylation to a higher extent than Ser-73 phosphorylation and that adiponectin stimulated Ser-73 phosphorylation longer than Ser-63 phosphorylation. This indicates that Ser-63 is more susceptible to both phoshporylation and dephosphorylation than Ser-73 during adiponectin treatment. Therefore, Ser-63 phoshporylation may play a major role in the immediate control of c-Jun activation by adiponectin, and Ser-73 phosphorylation may be important in the sustained activation of c-Jun.

Inhibition of constitutive STAT3 activation by f- and g-adiponectin

We examined the effect of f- and g-adiponectin on STAT3 activation using an EMSA. Constitutive STAT3 activation was detected in DU145 and HepG2 cells (Fig. 4A). Both f- and g-adiponectin inhibited constitutive STAT3 activation drastically in these cell lines (Figs. 4B and C). STAT3 activation returned to the original level in DU145 cells and began to rise in HepG2 cells within 60 min after adding f- or g-adiponectin. Neither f- nor g-adiponectin influenced STAT3 activation in any of the other cell lines (data not shown).

Discussion

Adiponectin is a major adipose cytokine that ameliorates metabolic disorders, such as obesity and diabetes mellitus [9,10]. Adiponectin may also influence cancer pathogenesis [17,18]. Obesity is associated with certain types of cancer, including prostate cancer [45–47] and hepatocellular carcinoma [48]. In this study, we employed prostate cancer cells, hepatocellular carcinoma cells, and immortalized myoblasts, and showed for the first time that adiponectin modulates the activation of JNK and STAT3 pathways. This suggests that JNK and STAT3 may be involved in adiponectin-mediated, metabolic alteration, and that adiponectin may influence the pathophysiology of these obesity-associated cancers by acting on tumor cells directly mediated by these signaling pathways.

Both f-adiponectin (consisting mainly of the HMW form) and g-adiponectin stimulated JNK activation (Fig. 2) and c-Jun phosphorylation (Fig. 3) in all the cell lines examined, and inhibited STAT3 activation where it was constitutively activated in DU145 and HepG2 cells (Fig. 4). As mentioned above, extensive evidence indicates that distinct forms of adiponectin differentially activate such signaling molecules as AMPK and NF-κB to mediate diverse biological functions among different cell types. Therefore, JNK and STAT 3 may be involved in pivotal pathways for any forms of adiponectin in universal biological functions, while such signaling pathways as AMPK and NF-κB may determine specific roles of the distinct forms of adiponectin in certain functions.

Adiponectin exerts antidiabetic effects by enhancing insulin action through such signaling pathways as the AMPK pathway in muscle and liver as stated above [10,23,24]. We demonstrated that adiponectin activated JNK remarkably in C2C12 myoblasts and hepatocellular carcinoma HepG2 cells (Fig. 2), suggesting the possible involvement of JNK in adiponectin-mediated, antidiabetic effects. It is interesting to note that JNK activity is abnormally elevated in muscle, liver, and adipose tissue in obesity and insulin resistance [36]. Highly activated JNK in obesity may mediate a negative feedback loop to maintain normal metabolic homeostasis by adiponectin: activated JNK may sensitize muscle cells and hepatocytes to adiponectin, enhancing its antidiabetic effects. Furthermore, JNK activation accompanied STAT3 inhibition in HepG2 cells during adiponectin treatment (Fig. 4). Thus, STAT3 inhibition concomitant with JNK activation may be important in antidiabetic action of adiponectin in hepatocytes.

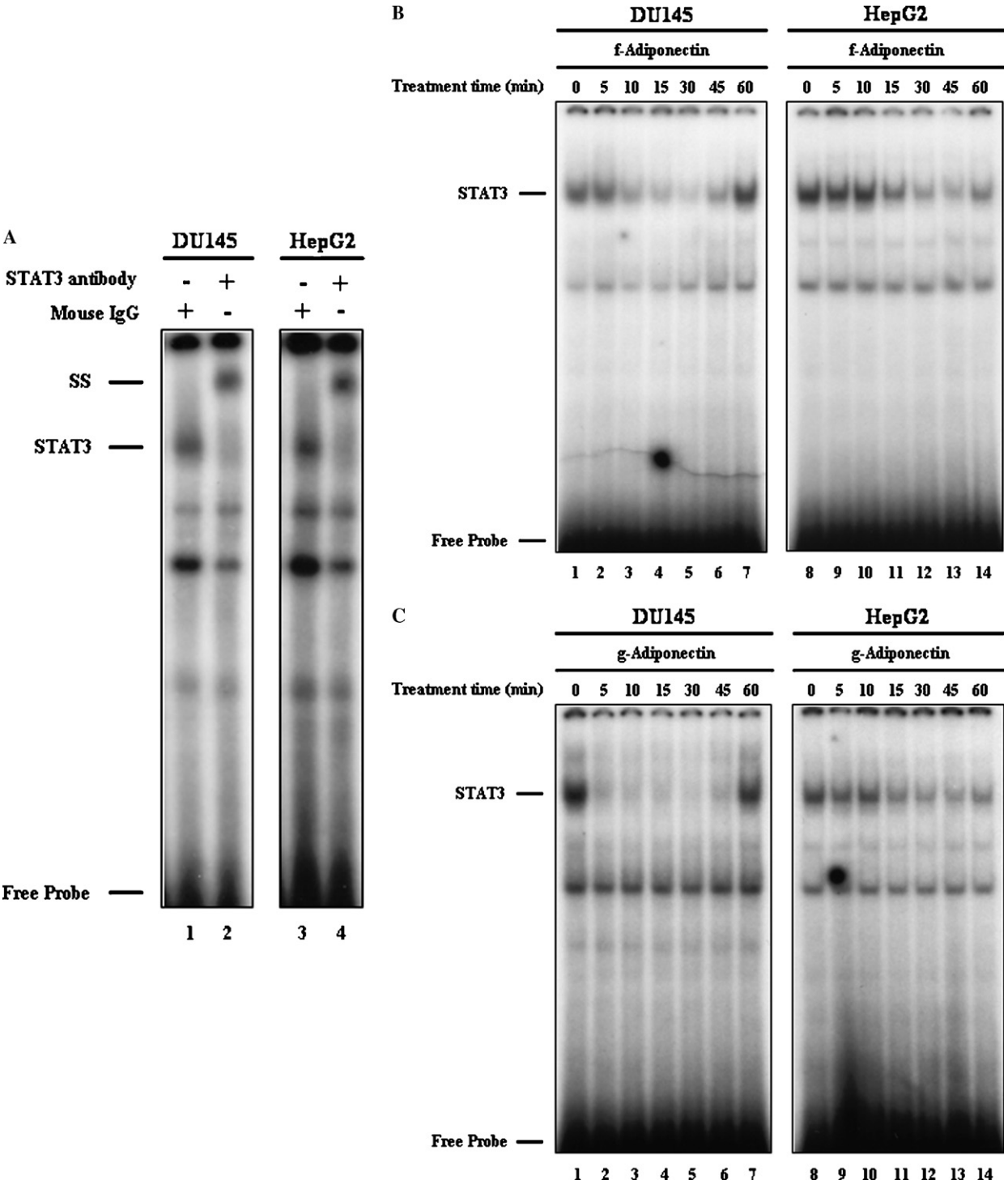


Fig. 4. (A) STAT3 is constitutively activated in DU145 and HepG2 cells. Prostate cancer DU145 cells and hepatocellular carcinoma HepG2 cells were serum-starved for 24 h, and cell lysates were prepared. Cell lysates (10 μg protein) were subjected to the electromobility shift assay using ^{32}P-end-labeled M67-SIE as a probe. The STAT3–DNA complex (STAT3) was observed and supershifted (SS) by the anti-STAT3 antibody. (B) f-Adiponectin inhibits STAT3 DNA binding activity in prostate cancer DU145 cells and hepatocellular carcinoma HepG2 cells. Prostate cancer DU145 cells and hepatocellular carcinoma HepG2 cells were deprived of serum for 24 h and incubated in serum-free medium (lanes 1 and 8) or serum-free medium containing 1.0 μg/ml f-adiponectin (lanes 2–7 and 9–14) for various periods up to 60 min. Cell lysates (10 μg protein) were subjected to the electromobility shift assay using ^{32}P-end-labeled M67-SIE as a probe. STAT3, STAT3–DNA complex. (C) g-Adiponectin inhibits STAT3 DNA binding activity in prostate cancer DU145 cells and hepatocellular carcinoma HepG2 cells. Prostate cancer DU145 cells and hepatocellular carcinoma HepG2 cells were deprived of serum for 24 h and incubated in serum-free medium (lanes 1 and 8) or serum-free medium containing 1.0 μg/ml g-adiponectin (lanes 2–7 and 9–14) for various periods up to 60 min. Cell lysates (10 μg protein) were subjected to the electromobility shift assay using ^{32}P-end-labeled M67-SIE as a probe. STAT3, STAT3–DNA complex.

JNK plays a crucial role in various cancers as well [35]. We demonstrated that adiponectin stimulated JNK activation (Fig. 2) and c-Jun phosphorylation (Fig. 3) in all the four cancer cell lines examined, including prostate cancer DU145, PC-3, and LNCaP-FGC cells and hepatocellular carcinoma HepG2 cells (Figs. 2 and 3). Furthermore, adiponectin inhibited STAT3 activation in DU145 and HepG2 cells (Figs. 4B and C), in which STAT3 was constitutively activated (Fig. 4A). Therefore, adiponectin acts on these cancer cells directly and may regulate their function through modulation of JNK and STAT3. It should be noted that constitutive STAT3 activation induces malignant transformation and stimulates cancer progression including cell growth promotion [40]. Thus, inhibition of constitutive STAT3 activation is considered to be a therapeutic intervention for cancer [40]. Therefore, adiponectin may also be therapeutic through STAT3 inactivation. Further investigation is ongoing in our laboratory to address potential roles of adiponectin in cancer pathogenesis.

In conclusion, JNK and STAT3 may play a role in adiponectin suppression of metabolic syndrome, and adiponectin may influence the pathogenesis of prostate cancer and hepatocellular carcinoma by acting on tumor cells directly via these signaling molecules. Since obesity is associated with some types of cancer, including prostate cancer [45–47] and hepatocellular carcinoma [48], adiponectin, as well as JNK and STAT3, may be molecular mediators between obesity and its associated cancers and could be their common therapeutic targets.

Acknowledgment

We thank Dr. Daniel H. Maloney for a critical review of the manuscript.

References

[1] K. Maeda, K. Okubo, I. Shimomura, T. Funahashi, Y. Matsuzawa, K. Matsubara, cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1), Biochem. Biophys. Res. Commun. 221 (1996) 286–289.

[2] E. Hu, P. Liang, B.M. Spiegelman, AdipoQ is a novel adipose-specific gene dysregulated in obesity, J. Biol. Chem. 271 (1996) 10697–10703.

[3] P.E. Scherer, S. Williams, M. Fogliano, G. Baldini, H.F. Lodish, A novel serum protein similar to C1q, produced exclusively in adipocytes, J. Biol. Chem. 270 (1995) 26746–26749.

[4] Y. Arita, S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi, Y. Matsuzawa, Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity, Biochem. Biophys. Res. Commun. 257 (1999) 79–83.

[5] M. Cnop, P.J. Havel, K.M. Utzschneider, D.B. Carr, M.K. Sinha, E.J. Boyko, B.M. Retzlaff, R.H. Knopp, J.D. Brunzell, S.E. Kahn, Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex, Diabetologia 46 (2003) 459–469.

[6] K. Hotta, T. Funahashi, Y. Arita, M. Takahashi, M. Matsuda, Y. Okamoto, H. Iwahashi, H. Kuriyama, N. Ouchi, K. Maeda, M. Nishida, S. Kihara, N. Sakai, T. Nakajima, K. Hasegawa, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Hanafusa, Y. Matsuzawa, Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1595–1599.

[7] M. Kumada, S. Kihara, S. Sumitsuji, T. Kawamoto, S. Matsumoto, N. Ouchi, Y. Arita, Y. Okamoto, I. Shimomura, H. Hiraoka, T. Nakamura, T. Funahashi, Y. Matsuzawa, Association of hypoadiponectinemia with coronary artery disease in men, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 85–89.

[8] Y. Iwashima, T. Katsuya, K. Ishikawa, N. Ouchi, M. Ohishi, K. Sugimoto, Y. Fu, M. Motone, K. Yamamoto, A. Matsuo, K. Ohashi, S. Kihara, T. Funahashi, H. Rakugi, Y. Matsuzawa, T. Ogihara, Hypoadiponectinemia is an independent risk factor for hypertension, Hypertension 43 (2004) 1318–1323.

[9] J. Fruebis, T.S. Tsao, S. Javorschi, D. Ebbets-Reed, M.R. Erickson, F.T. Yen, B.E. Bihain, H.F. Lodish, Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice, Proc. Natl. Acad. Sci. USA 98 (2001) 2005–2010.

[10] A.H. Berg, T.P. Combs, X. Du, M. Brownlee, P.E. Scherer, The adipocyte-secreted protein Acrp30 enhances hepatic insulin action, Nat. Med. 7 (2001) 947–953.

[11] N. Maeda, I. Shimomura, K. Kishida, H. Nishizawa, M. Matsuda, H. Nagaretani, N. Furuyama, H. Kondo, M. Takahashi, Y. Arita, R. Komuro, N. Ouchi, S. Kihara, Y. Tochino, K. Okutomi, M. Horie, S. Takeda, T. Aoyama, T. Funahashi, Y. Matsuzawa, Diet-induced insulin resistance in mice lacking adiponectin/ACRP30, Nat. Med. 8 (2002) 731–737.

[12] N. Ouchi, M. Ohishi, S. Kihara, T. Funahashi, T. Nakamura, H. Nagaretani, M. Kumada, K. Ohashi, Y. Okamoto, H. Nishizawa, K. Kishida, N. Maeda, A. Nagasawa, H. Kobayashi, H. Hiraoka, N. Komai, M. Kaibe, H. Rakugi, T. Ogihara, Y. Matsuzawa, Association of hypoadiponectinemia with impaired vasoreactivity, Hypertension 42 (2003) 231–234.

[13] C. Mantzoros, E. Petridou, N. Dessypris, C. Chavelas, M. Dalamaga, D.M. Alexe, Y. Papadiamantis, C. Markopoulos, E. Spanos, G. Chrousos, D. Trichopoulos, Adiponectin and breast cancer risk, J. Clin. Endocrinol. Metab. 89 (2004) 1102–1107.

[14] Y. Miyoshi, T. Funahashi, S. Kihara, T. Taguchi, Y. Tamaki, Y. Matsuzawa, S. Noguchi, Association of serum adiponectin levels with breast cancer risk, Clin. Cancer Res. 9 (2003) 5699–5704.

[15] E. Petridou, C. Mantzoros, N. Dessypris, P. Koukoulomatis, C. Addy, Z. Voulgaris, G. Chrousos, D. Trichopoulos, Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece, J. Clin. Endocrinol. Metab. 88 (2003) 993–997.

[16] L. Dal Maso, L.S. Augustin, A. Karalis, R. Talamini, S. Franceschi, D. Trichopoulos, C.S. Mantzoros, C. La Vecchia, Circulating adiponectin and endometrial cancer risk, J. Clin. Endocrinol. Metab. 89 (2004) 1160–1163.

[17] T. Yokota, K. Oritani, I. Takahashi, J. Ishikawa, A. Matsuyama, N. Ouchi, S. Kihara, T. Funahashi, A.J. Tenner, Y. Tomiyama, Y. Matsuzawa, Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages, Blood 96 (2000) 1723–1732.

[18] E. Brakenhielm, N. Veitonmaki, R. Cao, S. Kihara, Y. Matsuzawa, B. Zhivotovsky, T. Funahashi, Y. Cao, Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated

endothelial cell apoptosis, Proc. Natl. Acad. Sci. USA 101 (2004) 2476–2481.
[19] Y. Nakano, T. Tobe, N.H. Choi-Miura, T. Mazda, M. Tomita, Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma, J. Biochem. (Tokyo) 120 (1996) 803–812.
[20] U.B. Pajvani, X. Du, T.P. Combs, A.H. Berg, M.W. Rajala, T. Schulthess, J. Engel, M. Brownlee, P.E. Scherer, Structure–function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity, J. Biol. Chem. 278 (2003) 9073–9085.
[21] K. Kishida, H. Nagaretani, H. Kondo, H. Kobayashi, S. Tanaka, N. Maeda, A. Nagasawa, T. Hibuse, K. Ohashi, M. Kumada, H. Nishizawa, Y. Okamoto, N. Ouchi, K. Maeda, S. Kihara, T. Funahashi, Y. Matsuzawa, Disturbed secretion of mutant adiponectin associated with the metabolic syndrome, Biochem. Biophys. Res. Commun. 306 (2003) 286–292.
[22] T.S. Tsao, H.E. Murrey, C. Hug, D.H. Lee, H.F. Lodish, Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30), J. Biol. Chem. 277 (2002) 29359–29362.
[23] T. Yamauchi, J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N.H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai, T. Kadowaki, Cloning of adiponectin receptors that mediate antidiabetic metabolic effects, Nature 423 (2003) 762–769.
[24] T. Yamauchi, J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B.B. Kahn, T. Kadowaki, Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase, Nat. Med. 8 (2002) 1288–1295.
[25] T.S. Tsao, E. Tomas, H.E. Murrey, C. Hug, D.H. Lee, N.B. Ruderman, J.E. Heuser, H.F. Lodish, Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. Different oligomers activate different signal transduction pathways, J. Biol. Chem. 278 (2003) 50810–50817.
[26] N. Ouchi, H. Kobayashi, S. Kihara, M. Kumada, K. Sato, T. Inoue, T. Funahashi, K. Walsh, Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells, J. Biol. Chem. 279 (2004) 1304–1309.
[27] H. Kobayashi, N. Ouchi, S. Kihara, K. Walsh, M. Kumada, Y. Abe, T. Funahashi, Y. Matsuzawa, Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin, Circ. Res. 94 (2004) e27–e31.
[28] H. Chen, M. Montagnani, T. Funahashi, I. Shimomura, M.J. Quon, Adiponectin stimulates production of nitric oxide in vascular endothelial cells, J. Biol. Chem. 278 (2003) 45021–45026.
[29] N. Ouchi, S. Kihara, Y. Arita, Y. Okamoto, K. Maeda, H. Kuriyama, K. Hotta, M. Nishida, M. Takahashi, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Funahashi, Y. Matsuzawa, Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway, Circulation 102 (2000) 1296–1301.
[30] B. Derijard, M. Hibi, I.H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, R.J. Davis, JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain, Cell 76 (1994) 1025–1037.
[31] M. Hibi, A. Lin, T. Smeal, A. Minden, M. Karin, Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain, Genes Dev. 7 (1993) 2135–2148.
[32] J.M. Kyriakis, P. Banerjee, E. Nikolakaki, T. Dai, E.A. Rubie, M.F. Ahmad, J. Avruch, J.R. Woodgett, The stress-activated protein kinase subfamily of c-Jun kinases, Nature 369 (1994) 156–160.
[33] J. Raingeaud, S. Gupta, J.S. Rogers, M. Dickens, J. Han, R.J. Ulevitch, R.J. Davis, Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine, J. Biol. Chem. 270 (1995) 7420–7426.
[34] R.J. Davis, Signal transduction by the c-Jun N-terminal kinase, Biochem. Soc. Symp. 64 (1999) 1–12.
[35] R.J. Davis, Signal transduction by the JNK group of MAP kinases, Cell 103 (2000) 239–252.
[36] J. Hirosumi, G. Tuncman, L. Chang, C.Z. Gorgun, K.T. Uysal, K. Maeda, M. Karin, G.S. Hotamisligil, A central role for JNK in obesity and insulin resistance, Nature 420 (2002) 333–336.
[37] U. Ozcan, Q. Cao, E. Yilmaz, A.H. Lee, N.N. Iwakoshi, E. Ozdelen, G. Tuncman, C. Gorgun, L.H. Glimcher, G.S. Hotamisligil, Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes, Science 306 (2004) 457–461.
[38] D.E. Levy, J.E. Darnell Jr., Stats: transcriptional control and biological impact, Nat. Rev. Mol. Cell Biol. 3 (2002) 651–662.
[39] D.S. Aaronson, C.M. Horvath, A road map for those who don't know JAK-STAT, Science 296 (2002) 1653–1655.
[40] T. Bowman, R. Garcia, J. Turkson, R. Jove, STATs in oncogenesis, Oncogene 19 (2000) 2474–2488.
[41] Q. Gao, M.J. Wolfgang, S. Neschen, K. Morino, T.L. Horvath, G.I. Shulman, X.Y. Fu, Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation, Proc. Natl. Acad. Sci. USA 101 (2004) 4661–4666.
[42] M. Onuma, J.D. Bub, T.L. Rummel, Y. Iwamoto, Prostate cancer cell-adipocyte interaction: leptin mediates androgen-independent prostate cancer cell proliferation through c-Jun NH2-terminal kinase, J. Biol. Chem. 278 (2003) 42660–42667.
[43] Y. Iwamoto, Y.E. Chin, X. Peng, X.Y. Fu, Identification of a membrane-associated inhibitor(s) of epidermal growth factor-induced signal transducer and activator of transcription activation, J. Biol. Chem. 273 (1998) 18198–18204.
[44] E. Shaulian, M. Karin, AP-1 as a regulator of cell life and death, Nat. Cell Biol. 4 (2002) E131–E136.
[45] C. Rodriguez, A.V. Patel, E.E. Calle, E.J. Jacobs, A. Chao, M.J. Thun, Body mass index, height, and prostate cancer mortality in two large cohorts of adult men in the United States, Cancer Epidemiol. Biomarkers Prev. 10 (2001) 345–353.
[46] C.L. Amling, C.J. Kane, R.H. Riffenburgh, J.F. Ward, J.L. Roberts, R.S. Lance, P.A. Friedrichs, J.W. Moul, Relationship between obesity and race in predicting adverse pathologic variables in patients undergoing radical prostatectomy, Urology 58 (2001) 723–728.
[47] Y. Furuya, S. Akimoto, K. Akakura, H. Ito, Smoking and obesity in relation to the etiology and disease progression of prostate cancer in Japan, Int. J. Urol. 5 (1998) 134–137.
[48] J.M. Regimbeau, M. Colombat, P. Mognol, F. Durand, E. Abdalla, C. Degott, F. Degos, O. Farges, J. Belghiti, Obesity and diabetes as a risk factor for hepatocellular carcinoma, Liver Transpl. 10 (2004) S69–S73.