Arch Pharm Res Vol 28, No 11, 1263-1269, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Adiponectin Induces Growth Arrest and Apoptosis of MDA-MB-231 Breast Cancer Cell

Jee Hyun Kang*, Yoon Young Lee[1]*, Byung Yeon Yu, Beom-Seok Yang[2], Kyung-Hwan Cho[1], Do Kyoung Yoon[1], and Yong Kyun Roh[3]

Department of Family Medicine, College of Medicine, Konyang University. Daejon 320-718, Korea, [1]Department of Family Medicine, College of Medicine, Korea University. Seoul 136-705, Korea, [2]Biomedical Center, KIST, Seoul 136-791, Korea, and [3]Department of Family Medicine, College of Medicine, Hallym University, Seoul 150-071, Korea

(Received September 23, 2005)

Recently, it was reported that reduction in serum adiponectin levels is correlated with the incidence of breast cancer. As an effort to explain this, we screened various human breast cancer cell lines to identify those in which proliferation is directly controlled by adiponectin. Among the five tested cell lines, proliferation of MDA-MB-231 cancer cell was significantly suppressed by adiponectin within the range of physiological concentration. Furthermore, prolonged adiponectin treatment caused cell growth arrest and even apoptosis of MDA-MB-231. This result is the first to show that adiponectin can directly control cancer cell growth and provides a rationale for the theory that reduction in plasma adiponectin levels could be a risk factor for breast cancer.

Key words: Adiponectin, Breast cancer, MDA-MB-231, Growth arrest, Apoptosis

INTRODUCTION

Adiponectin is secreted from adipocytes and has been identified as one of the adipokines, a group that includes leptin and resistin. It exists at a relatively high concentration in serum, normally in the microgram per milliliter range. Other adipokines, such as leptin and resistin, are normally at serum levels in the nanogram per milliliter range (Fantuzzi, 2005). Adiponectin forms multimers and consists of two domains, the globular domain with a similarity to TNF-alpha, and a rod- like domain with a homology to the collagen sequence (Scherer *et al.*, 1995; Maeda *et al.*, 1996; Waki *et al.*, 2005).

The physiological role and biochemical function of adiponectin has been studied extensively in recent years. It has been established that plasma adiponectin concentration is inversely correlated with the incidence of obesity, type 2 diabetes, and cardiovascular disease (Matsuzawa *et al.*, 1999; Hu *et al.*, 1996; Arita *et al.*, 1999; Weyer *et al.*, 2001). In animal models, administration of adiponectin suppressed obesity, glucose tolerance, and athrosclerosis. This data suggests that adiponectin seems to have a protective activity against metabolic disorders (Yamauchi *et al.*, 2003; Okamoto *et al.*, 2002). Adiponectin was shown to activate AMP-activated protein kinase (AMPK), a key regulator for energy metabolism (Fruebis *et al.*, 2001; Yamauchi *et al.*, 2002). In addition, adiponectin proposed to induce the anti-inflammatory cytokine IL-10 and to reduce ICAM-1 induction by TNF-alpha (kumada *et al.*, 2004; Wolf *et al.*, 2004; Ouchi *et al.*, 1999). Based on these studies, adiponectin was proposed to possess anti-inflammatory activity (Fantuzzi, 2005). Although various physiological and biochemical functions have been attributed to adiponectin, its precise molecular working mechanism is still largely unknown.

Interestingly, recent several case-control studies have shown that decreased adiponectin levels are associated with the incidence of various human cancers such as endometrial, breast, prostate, and gastric cancers (Miyoshi *et al.*, 2003; Mantzoros *et al.*, 2004; Petridou *et al.*, 2003; Dal Maso *et al.*, 2004; Rose *et al.*, 2004; Goktas *et al.*, 2005; Ishikawa *et al.*, 2005). Although its underlying biological mechanism is still obscure, it has been shown in a mouse tumor model that adiponectin significantly

*These authors contributed equally to this work.
Correspondence to: Yong Kyun Roh, Department of Family Medicine, Kangnam Sacred Heart Hospital, Hallym University, College of Medicine. 948-1 Daelim-dong, Youngdungpo-ku, Seoul 150-071, Korea
Tel: 82-2-829-5270, Fax: 82-2-832-2839
E-mail: rohyk@hallym.ac.kr

inhibits primary tumor growth and it is associated with decreased neovascularization (Brakenhielm *et al.*, 2004).

In this paper, we examined whether adiponectin could directly affect breast cancer cell proliferation. From screening various breast cancer cell lines, we found that the growth of MDA-MB-231 cells is significantly affected by adiponectin, which caused growth arrest as well as apoptosis of the cancer cells.

MATERIALS AND METHODS

Construction of adiponectin expression plasmid

The baculoviral expression vector of glutathione-*S*-transferase (GST)-tagged human adiponectin was constructed using the pBacPAK8 vector (Clontech U.S.A.). The baculoviral expression vector of GST-tagged adiponectin was made by subcloning whole *c*-DNA of human adiponectin (purchased from Resgen) into the Bam H1-EcoR1 site of GST-tagged pBacPAK8 vector. All c-DNA fragments were obtained by PCR and were extensively sequenced for the verification of correct sequence.

Expression and purification of recombinant adiponectin

The generated baculoviral expression vector plasmid DNA of GST-adiponectin was transfected into sf9 cells using a baculovirus generation kit purchased from Clontech according to the manufacturer's manual. The viral stock was amplified to a titer of approximately 10^8 pfu /mL. These sf9 cells were infected with MOI of 10 and left for 48 h before harvest. Infected sf9 cells from 400 mL cultures were suspended in 20 mL of lysis buffer consisting of 20 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 1 mM DTT, 10 mM NaF, 0.1 mM EDTA, 0.1 M sodium vanadate, 0.02% IGE-PAL (Sigma), and a proteinase inhibitor cocktail tablet (Roche). In this buffer, the cells were lysed by sonication for 1 min. The supernatant of the lysate was applied to a 1 mL bed volume of glutathione-agarose column (Amersham) and washed with 20 mM Tris-HCl buffer (pH8.0) and with 0.15 M NaCl buffer. The bound proteins were eluted with 10 mL of washing buffer containing 20 mM reduced glutathione and subsequently concentrated to 1 mL using a Vivaspin concentrator. The concentrated sample was applied to a HiLoad 16/60 Superdex 200 prep grade column. The column had been pre-equilibrated with a buffer of 20 mM Tris-Hcl (pH 8.0) containing 0.15 M NaCl, 1 mM DTT, 10 mM NaF, and 0.1 mM EDTA. The sample was separated with a flow rate of 0.5 mL/min using an FPLC system.

Cell culture

Human breast cancer cells were obtained from the Korea Research Institute of Bioscience and Biotechnology (KRIBB) and from the Cancer Cell Bank of Seoul National University's medical college. Cells were grown in RPMI with 10% fetal bovine serum at 37°C under 5% CO_2 in a humid atmosphere.

MTT assay

Cells in a 96 well plate were incubated with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Roche, Germany) solution for 4 h. After purple formazan salt crystals formed, formazan soliblization solution (1% SDS in 0.01 M HCl) was added to each well to dissolve these crystals. After 5 min more incubations, the formazan crystals were solubilized. The resulting colored solution was quantified by measuring absorbance at 570 nm using a spectrophotometer.

Cell cycle analysis

MDA-MB-231 cells were plated at a density of 1×10^6 cells/well on six-well plates. After treatment with adiponectin for various periods of time, both detached and attached cells were collected into flow cytometry tubes and centrifuged at 1000 rpm for 5 min to obtain a cell pellet. The cells were suspended in a solution of PBS/EDTA and an equal volume of ethanol was added to the cells, which were then incubated for 30 min at room temperature. Cells were collected by centrifugation. For cell cycle analysis, the ethanol-fixed cells were stained with propidium iodide (PI) in the presence of RNase A and then analyzed by fluorescence-activated cell sorter (FACS). In each analysis, 10,000 events were recorded.

Apoptosis assay

For analyzing apoptosis, FITC- conjugated annexin V binding and PI staining were performed using a kit from Promega according to the manufacturer's manual. MB-231 cells were plated at a density of 1×10^6 cells/well on six-well plates and cell apoptosis was induced by adiponectin treatment for 48 h. Both detached and attached cells were collected for FITC-conjugated annexin V and PI staining. Early and late apoptotic cell populations were visualized by constructing a dot-plot with the aid of FACS. The FL1 channel was used to detect annexin-V-FITC staining and the FL2 channel was used for PI staining.

RESULTS

Purification of recombinant human adiponectin

We expressed the human full-length adiponectin protein as GST fusion in sf9 cells by baculoviral infection. The expressed GST-adiponectin fusion protein was purified using glutathione agarose affinity chromatography followed by gel filtration using FPLC. We obtained a purified

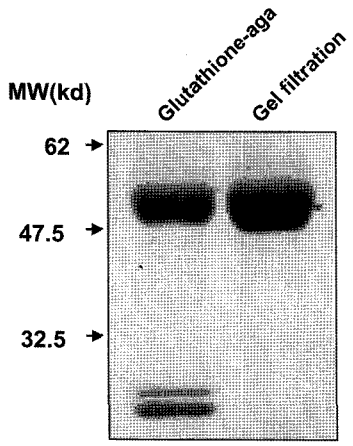

Fig. 1. Purification of GST-tagged recombinant human adiponectin. The expressed protein in sf9 cells was purified by glutathione agarose affinity chromatography and gel filtration chromatography using FPLC. The purified protein from each column step was loaded to 10% SDS-PAGE and stained with coomassie brilliant blue. MW; mol. Wt., kd; kilodalton.

protein with a molecular weight of approximately 52,000 daltons SDS-PAGE followed by coomassie brilliant blue staining revealed that the protein was at over 90% purity (Fig. 1). A Western blotting experiment was performed with an antibody specific to human adiponectin to confirm that the purified protein was adiponectin (data not shown).

Adiponectin inhibits MDA-MB-231 breast cancer cell proliferation

In previous reports, it was shown that a low adiponectin level in plasma is associated with high incidence of breast cancer in (Miyoshi *et al.*, 2003; Mantzoros *et al.*, 2004). To explain the reason for this, we hypothesized that adiponectin could directly suppress breast cancer cell proliferation. To test this hypothesis, we treated five human breast cancer cell lines with 30 ug/mL of recombinant GST-tagged adiponectin for two days and tested for inhibition of cell proliferation by MTT assay. Among the treated cancer cells, MDA-MB-231 cells showed a significant anti-proliferative response of about 40% inhibition by adiponectin. On the other hand, HS578T and SK-Br-3 cells showed a weak response of less than 15% inhibition (Fig. 2). In contrast, other non-specific GST-tagged recombinant proteins purified like GST-adiponectin showed no inhibitory effect on proliferation of these cells (data not shown). This suggests that adiponectin could directly inhibit the proliferation of some breast cancer cells such as MDA-MB-231.

We then treated MDA-MB-231 cells with concentrations of adiponectin ranging from 2.5 ug/mL to 30 ug/mL for two different time intervals; 24 h and 48 h. Previously, it was reported that normal serum adiponectin levels varied from 2 ug/mL to 20 ug/mL in screening of human populations

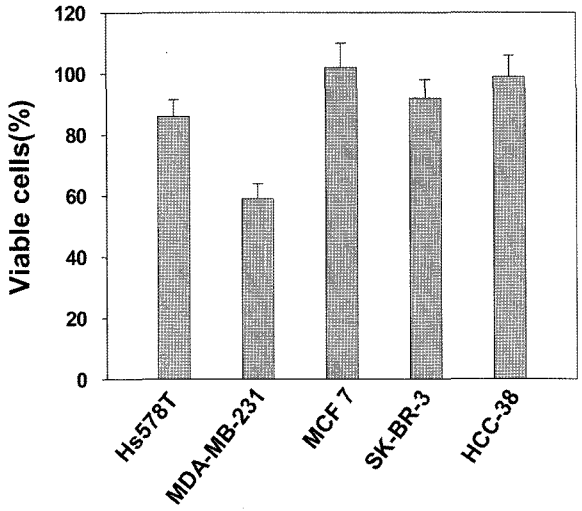

Fig. 2. Screening of human breast cancer cell lines for inhibition of proliferation by adiponectin. 5×10^3 cells of five human breast cancer cells were plated into 96- well plates and left overnight. Cells were treated with 30 ug/mL of GST-adiponectin for two days. The relative number of viable cells was estimated using MTT assay. A 100% value was assigned for the absorbance value of each cell with no adiponectin treatment. The values are shown as mean±S.D from three independent experiments.

(Miyoshi *et al.*, 2003). Therefore, the concentration range of adiponectin used in our experiments corresponds to naturally-occurring human physiological values. It has been reported that serum concentrations of adiponectin lower than 3-5 ug/mL significantly correlate with metabolic disorders as well as the incidence of breast cancer (Yamauchi *et al.*, 2001; Okamoto *et al.*, 2002; Miyoshi *et al.*, 2003). For both treatment time periods, dose-dependent inhibition of cell proliferation was observed (Fig. 3). With the treatment period of 24 h, we observed weak growth inhibition even at the maximum concentration of 30 ug/mL, with values not much greater than those observed for the untreated control. On the contrary, after 48 h of treatment, we could observe a significant growth inhibition of 40% at 30 ug/mL. These results imply that multi-step molecular events are necessary for adiponectin's function of switching MDA-MB-231 cells from a proliferative state to an impaired state of cell growth It appears that adiponectin requires more than 24 h to significantly affect MDA-MB-231 cell proliferation.

Adiponectin induces G0/G1 arrest and apoptosis of MDA-MB-321 cancer cell

To study the mechanism of antiproliferative activity by recombinant human adiponetin in detail, we analyzed the effects of adiponectin treatment on cell cycle distributions of MDA-MB-231 cells. Cells were treated with various concentration of recombinant human adiponetin for 24 h and subject to FACS analysis after PI staining of the

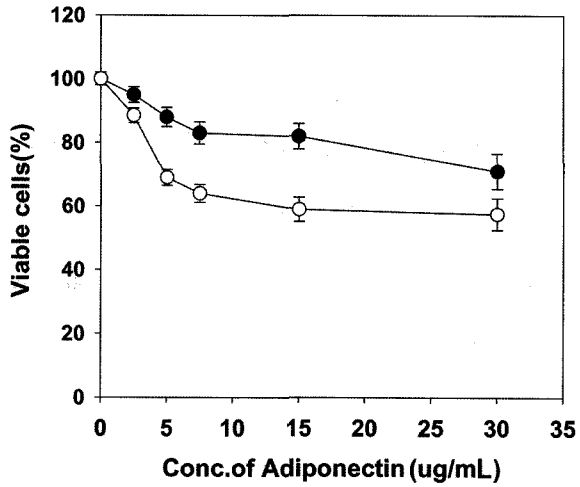

Fig. 3. Proliferation of MDA-MB-231 breast cancer cell is inhibited by adiponectin in a time and dose dependent manner. A similar experiment was done as in Fig. 2 using MDA-MB-231 cells treated with various concentrations of adiponectin for 24 h or for 48 h. A 100% value was assigned for the absorbance value from wells incubated for 24 h or 48 h with no adiponectin. filled circle; 24 h, open circle; 48 h. The values are shown as mean±S.D for n=3.

chromosomal DNA. In histograms of FACS analysis, untreated proliferative MDA-MB-231 cells showed cell cycle distributions of 51.95% in G1/G0, 10.49% in S, 37.57% in G2/M, and 3.37% in sub G1/G0 phase. However, after adiponectin treatment, G1/G0 and sub G1/G0 populations increased in an adiponectin-dose dependent manner. At 30 ug/mL of adiponectin, these populations reached a maximum of 60.1% for G1/G0 and 7.17% for subG1/G0. In contrast, the populations were reduced up to 8.14% in S phase and 31.76% in G2/M phase after treatment with 30 ug/mL of adiponectin (Fig. 4). These data indicates that adiponectin has an activity to arrest MDA-MB-231 cell growth in G0/G1.

We observed a significant reduction of viable cells when MDA-MB-231 cells were treated with adiponectin for 48 h

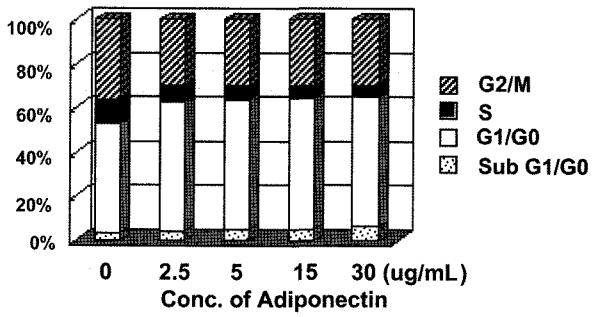

Fig. 4. Adiponectin arrests MDA-MB-231 cells in G0/G1. Proliferating MDA-MB-231 cells were treated with various concentrations of adiponectin for 24 h, fixed with ethanol, stained with PI. Cell cycle distributions were analysed by FACS analysis.

(Fig. 2). We also examined that adiponectin treatment for 48 h could induce apoptosis of MDA-MB-231 cells. Simultaneous staining of cells with annexin-V and PI dye made it possible to distinguish between early apoptotic cells (stained positive for annexin-V and negative for PI), and late apoptotic or necrotic cells (stained positive for both annexin-V and PI). In MDA-MB-231 control culture,

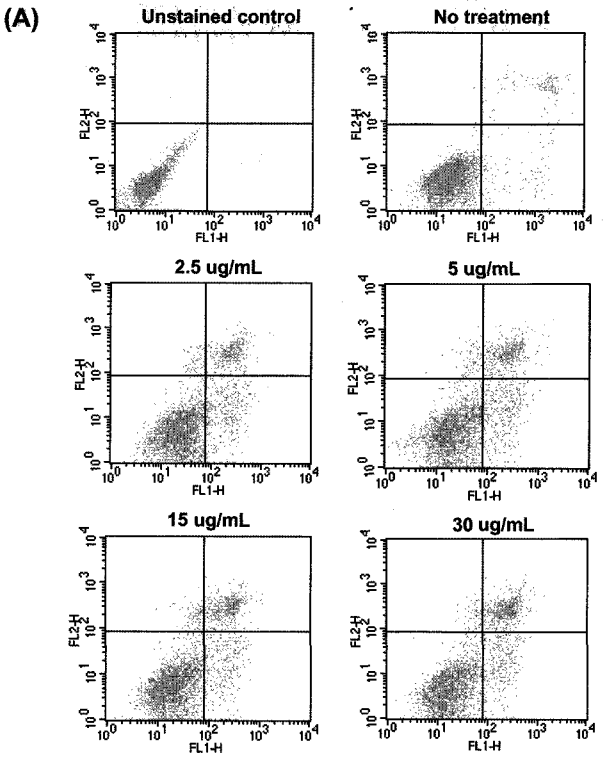

Fig. 5. Prolonged treatment of adiponectin causes apoptosis of MDA-MB-231 cells. MDA-MB-231 cells were treated with various concentrations of adiponectin for 48 h. The cells were then stained with both FITC-conjugated Annexin V and PI. FACS was used to obtain the stained cell population. (A) Histograms from FACS analysis at each adiponectin concentration. The FL1 channel was used to detect annexin-V-FITC staining and the FL2 channel was for PI staining. (B) Graphic presentation to show percentage of early apoptotic cells stained with Annexin-V-FITC (open circle) and late apoptotic or necrotic cells stained with both Annexin-V-FITC and PI (filled circle) at various concentrations of adiponectin.

2.47% of the total cells were early apoptotic cells and 2.93% were in the late stage of apoptosis or necrosis (Fig. 5). After 48 h of treatment with recombinant human adiponectin, the population of cancer cells in early apoptotic stage significantly increased. It reached a maximum value of approximately 12% at 2.5 ug/mL of adiponectin, then slightly decreased from this maximum value as the concentration of adiponectin became higher. On the other hand, the population in late apoptotic or necrotic stage continuously increased with the amount of treated adiponectin, giving a maximum value of about 27% at 30 ug/mL of adiponectin (Fig. 5). These results indicate that the persistent presence of adiponectin at concentrations of 10-30 ug/mL can cause significant MDA-MB-231 cell death through the apoptotic pathways. Note that 10-30 ug/mL of adiponectin is the concentration that is found frequently in the serum of healthy humans (Miyoshi *et al.*, 2003).

DISCUSSION

The preventive role of adiponectin against various human diseases has become widely recognized (Trujillo *et al.*, 2005). It is well established that low adiponectin levels in serum are directly correlated with the incidence of human metabolic disorders such as obesity, diabetes, and arthrosclerosis (Yamauchi *et al.*, 2001, Okamoto *et al.*, 2002). It has recently been proposed that adiponectin could play a positive role in suppressing cancer development. This hypothesis is based on the observation that adiponectin concentration was inversely proportional and independently associated with breast, endometrial, gastric, and prostate cancer in case control studies. It was also suggested that tumors arising in a person with low serum adiponectin levels are more likely to grow quickly and to be at a more advanced stage when diagnosed (Miyoshi *et al.*, 2003; Mantzoros *et al.*, 2004; Petridou *et al.*, 2003; Dal Maso *et al.*, 2004; Rose *et al.*, 2004; Goktas *et al.*, 2005; Ishikawa *et al.*, 2005).

Given the interesting correlation between adiponectin and cancer, studies to elucidate its precise underlying molecular mechanism have only just begun. There was a report to show that adiponectin induces endothelial cell apoptosis, thus exerting anti-angiogenic activity by preventing neovascularization in primary cancers (Brakenhielm *et al.*, 2004). Our study is the first one since then to establish that adiponectin is able to directly induce cancer cell growth arrest and apoptosis.

Though there is much evidence to support direct anticancer activity by adiponectin, it is still unknown just how adiponectin could show such anticancer activities. Adiponectin is a secreted protein and its cellular receptor has been identified only very recently (Yamauchi *et al.*, 2003). Cellular signalling of adiponectin inside cells through its receptor is as yet largely unknown. In this work, we show that adiponectin induces cancer cell growth arrest and apoptosis. However, this seems to occur only in some specific cell types such as MBA-MD-231 cells, suggesting that some specific cellular factor(s) might also be involved. We also observed that the induction of apoptosis by adiponectin takes a relatively long time. We think this might be because some complex cellular events triggered by adiponectin are also necessary to induce apoptosis.

An interesting finding from this work is that the direct anticancer activity of adiponectin could be detected in vitro at concentrations in the microgram per milliliter range in cell culture. Our results and others' reports showed that healthy human beings normally have 10-20 mg/mL of adiponectin circulating in their blood (Miyoshi *et al.*, 2003; Arita *et al.*, 1999; Weyer *et al.*, 2001). Therefore, our data is physiologically relevant and could explain why people with normal adiponectin levels in their blood are in less danger of developing cancers.

Serum adiponectin level is frequently found to be at a significantly reduced level in obese people (Matsuzawa *et al.*, 1999; Hu *et al.*, 1996). Obesity has been recognized as a serious health problem in developed countries because it is tightly linked to the development of such metabolic diseases as diabetes and cardiovascular disorders (Matsuzawa *et al.*, 1999; Hu *et al.*, 1996; Arita *et al.*, 1999; Weyer *et al.*, 2001). In addition to this, it has been proposed that obesity is related with the incidence of breast, endometrial, colon, and prostate cancer. Excess body weight is the cause of approximately 20% of all cancer deaths among women aged 50 years or older in United States (Calle *et al.*, 2003). Recent data strongly suggests that reduced blood levels of adiponectin might be the link to explain the correlation between obesity and cancer. Our results imply that high level of serum adiponectin could potentially contribute to decreased risk of breast cancer in obese women. Such information could provide a rationale for deducing some preventive or therapeutic strategies for human malignancy by increasing serum adiponectin levels among obese populations.

ACKNOWLEDGEMENT

This work was supported by Konyang University Myung Gok Research Fund of 2004 to Jee Hyun Kang.

REFERENCES

Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., Hotta, K., Shimomura, I., Nakamura, T., Miyaoka, K., Kuriyama, H., Nishida, M., Yamashita, S., Okubo, K., Matsubara, K., Muraguchi, M., Ohmoto, Y.,

Funahashi, T., and Matsuzawa Y., Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem. Biophys. Res. Commun.*, 257, 79-83 (1999).

Brakenhielm, E., Veitonmaki, N., Cao, R., Kihara, S., Matsuzawa, Y., Zhivotovsky, B., Funahashi, T., and Cao, Y., Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. *Proc. Nat. Acad. Sci. U.S.A.*, 101, 2476-2481 (2004).

Calle, E. E., Rodriguez, C., Walker-Thurmond, K., and Thun, M. J., Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N. Engl. J. Med.*, 348, 1625-1638 (2003).

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

Fantuzzi, G., Adipose tissue, adipokines, and inflammation. *J. Allergy. Clin. Immunol.*, 115, 911-919 (2005).

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

Goktas, S., Yilmaz, M. I., Caglar, K., Sonmez, A., Kilic, S., and Bedir, S., Prostate cancer and adiponectin. *Urology*, 65, 1168-1172 (2005).

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

Ishikawa, M., Kitayama, J., Kazama, S., Hiramatsu, T., Hatano, K., and Nagawa, H., Plasma adiponectin and gastric cancer. *Clin. Cancer Res.*, 11, 466-472 (2005).

Kumada, M., Kihara, S., Ouchi, N., Kobayashi, H., Okamoto, Y., Ohashi, K., Maeda, K., Nagaretani, H., Kishida, K., Maeda, N., Nagasawa, A., Funahashi, T., and Matsuzawa, Y., Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation*, 109, 2046-2049 (2004).

Maeda, K., Okubo, K., Shimomura, I., Funahashi, T., Matsuzawa, Y., and Matsubara, K., cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem. Biophys. Res. Commun.*, 221, 286-296 (1996).

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

Matsuzawa, Y., Funahashi, T., and Nakamura, T., Molecular, mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Ann. N. Y. Acad. Sci.*, 892, 146-154 (1999).

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

Okamoto, Y., Kihara, S., Ouchi, N., Nishida, M., Arita, Y., Kumada, M., Ohashi, K., Sakai, N., Shimomura, I., Kobayashi, H., Terasaka, N., Inaba, T., Funahashi, T., and Matsuzawa, Y., Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation*, 106, 2767-2770 (2002).

Ouchi, N., Kihara, S., Arita, Y., Maeda, K., Kuriyama, H., Okamoto, Y., Hotta, K., Nishida, M., Takahashi, M., Nakamura, T., Yamashita, S., Funahashi, T., and Matsuzawa, Y., Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*, 100, 2473-2476 (1999).

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

Rose, D. P., Komninou, D., and Stephenson, G. D., Obesity, adipocytokines, and insulin resistance in breast cancer. *Obes. Rev.*, 5, 153-165 (2004).

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

Trujillo, M. E. and Scherer, P. E., Adiponectin-journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J. Intern. Med.*, 257, 167-175 (2005).

Waki, H., Yamauchi, T., Kamon, J., Kita, S., Ito, Y., Hada, Y., Uchida, S., Tsuchida, A., Takekawa, S., and Kadowaki, T., Generation of globular fragment of adiponectin by leukocyte elastase secreted by monocytic cell line THP-1. *Endocrinology*, 146, 790-796 (2005).

Weyer, C., Funahashi, T., Tanaka, S., Hotta, K., Matsuzawa, Y., Pratley, R. E., and Tataranni, P. A., Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J. Clin. Endocrinol. Metab.*, 86, 1930-1935 (2001).

Wolf, A. M., Wolf, D., Rumpold, H., Enrich, B., and Tilg, H., Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem. Biophys. Res. Commun.*, 323, 630–635 (2004).

Yamauchi ,T., Kamon, J., Ito, Y., Tsuchida, A., Yokomizo, T., Kita, S., Sugiyama, T., Miyagishi, M., Hara, K., Tsunoda, M., Murakami, K., Ohteki, T., Uchida, S., Takekawa, S., Waki, H., Tsuno, N. H., Shibata, Y., Terauchi, Y., Froguel, P., Tobe, K., Koyasu, S., Taira, K., Kitamura, T., Shimizu, T., Nagai, R., and Kadowaki, T., Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*, 423, 762-769 (2003).

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S., Ueki, K., Eto, K., Akanuma, Y., Froguel, P., Foufelle, F., Ferre, P., Carling, D., Kimura, S., Nagai, R., Kahn, B. B., and Kadowaki, T., Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat. Med.*, 8, 1288-1295 (2002).

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M. L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P., and Kadowaki, T., The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat. Med.*, 7, 941-946 (2001).