Biochemical and Biophysical Research Communications 379 (2009) 288–292

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

RhoA induces expression of inflammatory cytokine in adipocytes

Yuki Nakayama [a], Ryutaro Komuro [b], Akiko Yamamoto [a], Yugo Miyata [a], Masaki Tanaka [b], Morihiro Matsuda [b], Atsunori Fukuhara [b,*], Iichiro Shimomura [a,b]

[a] Department of Organismal Biosystems, Graduate School of Frontier Biosciences, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 8 December 2008
Available online 25 December 2008

Keywords:
RhoA
ROCK
Adipocyte
PAI-1
MCP-1
TNF-α

ABSTRACT

Rho GTPase regulates actin cytoskeleton organization and assembly in many cell types, however, its significance in adipose tissue is not well characterized. Here, we demonstrate high RhoA activity in adipose tissues of C57BL/6J mice. To determine the effect of RhoA activation on 3T3-L1 cells, stable cell lines overexpressing G14VRhoA fused to destabilizing domain of FKBP12 (DD-G14VRhoA-L1) were generated. Treatment of DD-G14VRhoA-L1 cells with Shield1 following their differentiation into adipocytes, resulted in the appearance of thick cortical actin filaments, and increased the mRNA expression levels of plasminogen activator inhibitor type-1 (PAI-1) and monocyte chemoattractant protein-1 (MCP-1). The induction of PAI-1 and MCP-1 was inhibited by treatment with a Rho-associated kinase (ROCK) inhibitor, Y-27632. In 3T3-L1 adipocytes, tumor necrosis factor-α activated RhoA and increased mRNA expression of PAI-1 and MCP-1, and their treatment with Y-27632 partially inhibited these changes. The results indicate that RhoA-ROCK pathway induces inflammatory cytokine expression in adipocytes.

Obesity is considered a state of chronic inflammation of the adipose tissue with increased production of proinflammatory cytokines and chemokines such as tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor type-1 (PAI-1), and monocyte chemoattractant protein-1 (MCP-1) [1]. Furthermore, dysregulation of adipocytokines is linked to obesity-associated metabolic diseases [2,3]. TNF-α plays a significant role in the development of insulin resistance of obesity [4], and its neutralization reverses systemic insulin resistance [4], moreover, TNF-α accelerates the production of PAI-1, and MCP-1 [5]. PAI-1 is an inhibitor of tissue-type plasminogen activator and urokinase-type plasminogen activator [6]. PAI-1, released from adipose tissues [7], increases with visceral fat accumulation and also associates with inflammatory amplification [8]. MCP-1 is a potent chemotactic factor for monocytes, and plays an important role in atherogenesis [9]. A recent study revealed that MCP-1 is overexpressed in adipose tissue of obese mice, and such overexpression contributes to the macrophage infiltration, insulin resistance, and hepatic steatosis associated with obesity [10].

RhoA is a master regulator of various cellular processes such as cell migration, adhesion, cytokinesis, cell cycle progression, vesicular trafficking, and cytoskeletal regulation [11]. RhoA works as a molecular switch, shuttling between the GDP-bound inactive form and the GTP-bound active form [11]. RhoA also regulates gene expression through various transcription factors such as nuclear factor kappa-B (NF-κB) [11]. Recently, RhoA was also described to play an important role in differentiation of tissue-cultured adipocytes through Rho-associated kinase (ROCK) [12], a major effector of RhoA. However, very little is known about its physiological significance in differentiated adipose cells. In the present study, we found high RhoA activity in adipose tissues and investigated the role of RhoA-ROCK pathway in adipose cells.

Materials and methods

Antibodies, recombinant protein, and other reagents. Anti-RhoA antibody (Ab) was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), Anti-ezrin, radaxin, and moesin (ERM) and phosphorylated ERM Abs were from Cell Signaling Technology (Beverly, MA). Alexa Fluor 568 phalloidin were from Molecular Probes (Eugene, OR), 4′,6-diamino-2-phenylindole (DAPI) from Invitrogen (San Diego, CA), Shield1 from Clontech Laboratories Inc (Palo Alto, CA), recombinant human TNF-α from PeproTech Inc. (Rocky Hill, NJ), and Y-27632 from Calbiochem (San Diego, CA). Glutathione *S*-transferase-fused Rho-binding domain of rhotekin (GST-Rhotekin RBD) was kindly provided by Dr. Yoshimi Takai (Kobe University, Japan). Other reagents were purchased from Sigma–Aldrich Corporation (St. Louis, MO).

Animals. Male C57BL/6J mice were purchased from Clea Japan. The experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Osaka University.

* Corresponding author. Fax: +81 6 6879 3739.
E-mail address: fukuhara@imed2.med.osaka-u.ac.jp (A. Fukuhara).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.12.040

Cell culture. 3T3-L1 cells were obtained from ATCC (Rockville, MD), and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. 3T3-L1 preadipocytes were grown to confluence and induced to differentiate into adipocytes, as described previously [13].

GST pull-down assay for Rho GTPase and immunoblotting. RhoA activity was determined as described previously [14]. Briefly, 3T3-L1 cells were harvested or tissue was homogenized with lysis buffer, pelleted by centrifugation, and collected as cell lysates or tissue extract. Subsequently, RhoA-GTP was collected using GST-Rhotekin RBD. The resulting samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and bound proteins were detected by immunoblotting. For total cell lysates or tissue extract, 50 μg of protein were subjected to SDS–PAGE and immunoblotted by the indicated antibodies.

Isolation of SV and MA fraction: Epididymal fat pads from male C57BL/6J mice were excised and minced in Krebs–Ringer-bicarbonate–Hepes (*N*-2-hydroxyetylpiperazine-*N*′-ethanesulfonic acid) buffer containing 20 μM adenosine, and 4% (w/v) bovine serum albumin (BSA) (Calbiochem). Tissue suspensions were centrifuged at 500*g* for 5 min to remove erythrocytes and free leukocytes. Collagenase was added to 2 mg/ml and incubated at 37 °C for 20 min with shaking. The cell suspension was filtered through a 250 μm filter and then spun at 300*g* for 1 min to separate floating mature adipocytes fraction (MA) from the stromal vascular fraction (SV) pellet.

Generation of stable cell lines. The retroviral expression vector, pMSCV-HA-DD, was generated to express a fusion protein with the N-terminal HA epitope and destabilized domain (DD). pMSCV plasmid was purchased from Clontech. Human FKBP12 gene was cloned from cDNA from human adipocytes (Zen Bio, Inc., Research Triangle Park, NC), and F36V and L106P mutations were introduced using QuickChange II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). The cDNA fragments encoding full-length G14VRhoA (1–197 amino acids (aa)) were inserted into pMSCV-HA-DD. PlatE cells were transfected with pMSCV-HA-DD, or pMSCV-HA-DD-G14VRhoA, using Lipofectamine 2000. Viral supernatants were harvested after 48 h, and 3T3-L1 preadipocytes were infected with the concentrated supernatants supplemented with 10 μg/ml polybrene for 4 h at 37 °C, followed by selection with 2 μg/ml puromycin.

Immunofluorescence microscopy. 3T3-L1 cells were plated on microcover glass (Matsunami glass), fixed with 2–4% formaldehyde in phosphate buffered saline (PBS) for 10 min. The fixed cells were then treated with 0.2% Triton X-100 in PBS for 10 min. After being blocked in PBS containing 1% BSA for 1 h, the cells were incubated in the same buffer with Alexa Flour 568 Phalloidin for 1 h. The samples were then washed with tris-buffered saline three times, stained with DAPI and mounted in Vectashield (Vector Laboratories, Burlingame, CA). The samples were analyzed by Radiance 2100 confocal laser scanning microscope (Bio-Rad Laboratories Burlingame, CA) or BioZero microscope BZ-8000 (Keyence Corporation).

RNA isolation and quantitative-real-time RT-PCR. Total RNA from 3T3-L1 adipocytes and tissues were extracted using Sepasol-RNA I Super (Nacalai Tesk, Kyoto, Japan). The cDNA was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany). Quantitative real-time RT-PCR was performed using primers described previously [15].

Statistical analysis. All data were expressed as means ± SEM. Differences between groups were examined for statistical significance using the unpaired Student's *t*-test. Multiple comparisons were analyzed by one-way analysis of variance (ANOVA) followed by Tukey–Kramer post hoc test. A *P* value less than 0.05 denoted the presence of a statistically significant difference.

Results

RhoA activity is high in adipose tissues

To determine the significance of RhoA in adipose tissues, RhoA activity was analyzed in various tissue samples from C57BL/6J mice. RhoA expression levels were high in the brain, spleen, and epididymal white adipose tissues (WAT) (Fig. 1A). The pull-down assay using GST-Rhotekin RBD confirmed the high RhoA activity in the brain, spleen, and WAT. Next, total and phosphorylated ERM were assessed in the same samples. ERM proteins are known to be phosphorylated by ROCK, a direct target of Rho [16]. ERM expression levels were almost similar in all tissues, however, phosphorylated ERM levels were high in the kidney, spleen, WAT, and muscle. To further investigate the RhoA activity in adipocytes, adipose tissues were fractionated into the mature adipocyte fraction (MA, i.e., containing mature adipocytes), and stromal vascular fraction (SV, i.e., containing immature adipocytes, blood-derived cells, and endothelial cells). ERM levels were high in SV, whereas phosphorylated ERM was at high levels in MA and SV (Fig. 1B). These results indicate that RhoA activity is high in adipocytes.

Constitutive active RhoA induces PAI-1 and MCP-1 expression in adipocytes

To investigate the role of RhoA in adipocytes, stable 3T3-L1 cell lines overexpressing G14VRhoA fused to destabilizing domain (DD-G14VRhoA-L1) were generated. DD consists of mutated FKBP12 protein, rapidly and constitutively degraded when expressed in mammalian cells [17]. Treatment of DD-G14VRhoA-L1 cells with Shield1 [18] after their differentiation into adipocytes, resulted in induction of DD-G14VRhoA (Fig. 2A). Next, the actin cytoskeleton was analyzed to investigate the role of DD-G14VRhoA. In preadipocytes, Shield1 treatment enhanced condensation of actin stress fibers, but these changes were not observed in control cells overexpressing DD alone (Fig. 2B, left panel). After differentiation of DD-G14VRhoA-L1 cells into adipocytes, Shield1 treatment enhanced thick cortical actin filaments in DD-G14VRhoA-L1 cells, whereas these changes were not observed in control cells (Fig. 2B, right panel). Induction of DD-G14VRhoA in

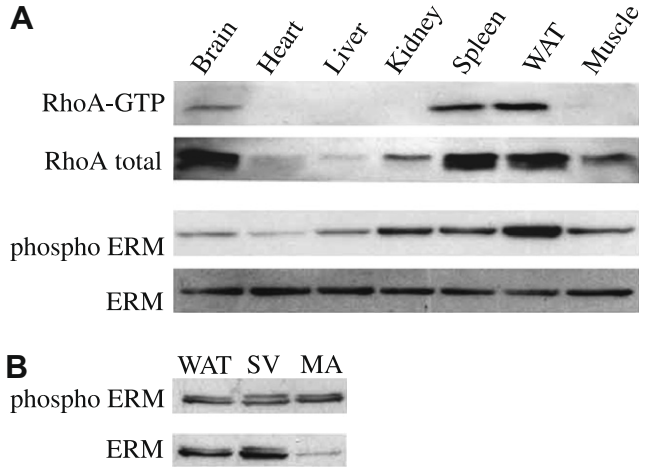

Fig. 1. RhoA and ERM activities in various tissues. (A) Fifty micrograms of each tissue extract was subjected to SDS–PAGE and Western blotting with anti-RhoA (RhoA total), anti-phospho-ERM and anti-ERM Abs. RhoA-GTP was isolated using GST-Rhotekin RBD, followed by SDS–PAGE and Western blotting with anti-RhoA Ab. (B) Fifty micrograms of total adipose tissues (WAT), stromal vascular fraction (SV), and mature adipocyte fraction (MA) were subjected to SDS–PAGE and Western blotting with anti-phospho-ERM and anti-ERM Abs.

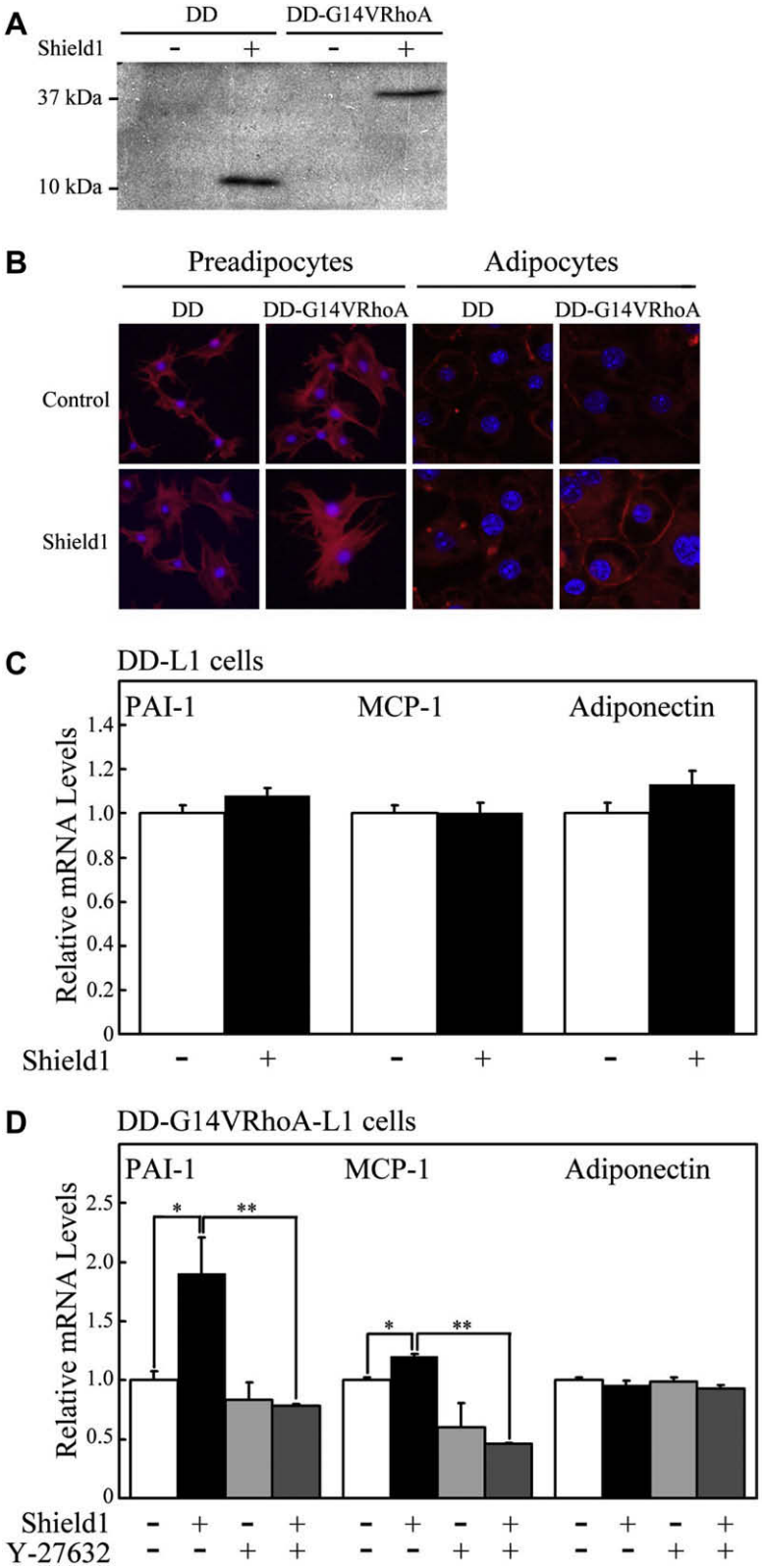

Fig. 2. Induction of active RhoA and changes in mRNA expressions of inflammatory cytokines in 3T3-L1 adipocytes. (A) After differentiation into adipocytes, 3T3-L1 cells expressing HA-DD (DD) and HA-DD-G14VRhoA (DD-G14VRhoA) were treated with or without 1 μM of Shield1 for 24 h, and cell lysates were subjected to SDS–PAGE and Western blotting with anti-HA Ab. (B) Undifferentiated (preadipocytes), and differentiated (adipocytes) 3T3-L1 cells expressing HA-DD (DD) or HA-DD-G14VRhoA (DD-G14VRhoA) were treated with or without 1 μM of Shield1 for 24 h, fixed, permeabilized, and stained with Alexa Fluor 568 phalloidin for F-actin (red), and DAPI for nucleus (blue). Magnification, preadipocytes (20×) and adipocytes (60×). (C,D) mRNA expression levels of PAI-1, MCP-1, and adiponectin in differentiated 3T3-L1 adipocytes expressing HA-DD (DD-L1 cells) or HA-DD-G14VRhoA (DD-G14VRhoA-L1 cells) with or without 1 μM Shield1 for 24 h. DD-G14VRhoA-L1 cells were treated with or without 30 μM Y-27632 for 24 h. The mRNA levels were quantified by real-time RT-PCR. Values are normalized to the level of cyclophilin mRNA and expressed as means ± SEM (n = 4–6).

differentiated adipocytes increased mRNA expression of PAI-1 and MCP-1 (Fig. 2D), while such change was not observed in control cells (Fig. 2C). Under this condition, Shield1 treatment did not affect lipid accumulation, as estimated by oil red-O staining (data not shown), adiponectin mRNA levels (Fig. 2E), or peroxisome proliferator-activated receptor γ mRNA levels (data not shown) in both cells. To elucidate the downstream signaling of RhoA, adipocytes were pretreated with Y-27632, a ROCK inhibitor, before the treatment with Shield1. This treatment restored Shield1-induced PAI-1 and MCP-1 expression (Fig. 2D). These results indicate that active RhoA induces PAI-1 and MCP-1 expression through ROCK in differentiated adipocytes.

RhoA-ROCK pathway affects TNF-α-induced production of proinflammatory cytokines

To identify the upstream factor of RhoA activation, pull-down assay was performed in 3T3-L1 adipocytes stimulated with TNF-α, dexamethasone, H_2O_2, glucose, or insulin. Treatment with H_2O_2, glucose, or insulin did not affect RhoA activity (data not shown), whereas TNF-α and dexamethasone activated RhoA within 4–6 h (Fig. 3A) as reported previously in other cell types [19,20]. Similar to the induction of G14VRhoA (Fig. 2E), TNF-α induced mRNA expressions of MCP-1 and PAI-1 (Fig. 3B), as described previously in 3T3-L1 adipocytes [5,15]. Y-27632 significantly inhibited the effects of TNF-α on MCP-1 and PAI-1 expression (Fig. 3B). These results indicate that the RhoA-ROCK pathway is involved, at least in part, in TNF-α induced expression of PAI-1 and MCP-1 in adipocytes.

Discussion

In the present study, we investigated the role of RhoA in 3T3-L1 adipocytes. In differentiated adipocytes, Furukawa et al. [21] reported that inactivation of ROCK impaired insulin signaling and insulin-stimulated glucose transport both *in vitro* and *in vivo*, using Y-27632 or adenoviral infection of dominant-negative form of ROCK. Unfortunately, mature adipocytes are very difficult to transfect with plasmid cDNAs, and transient adenoviral infection influences inflammatory responses in adipocytes. In the present study, we investigated for the first time the role of RhoA on inflammatory cytokine expression in differentiated adipocytes, using inducible cell lines expressing G14VRhoA fused to DD.

Several factors regulate PAI-1 and MCP-1 expression. For example, expression of MCP-1 is significantly decreased during differentiation of 3T3-L1 cells [22], and is induced by TNF-α, insulin, growth hormone, and IL-6 in 3T3-L1 adipocytes [22]. On the other hand, expression of PAI-1 is induced by TNF-α, insulin [23], oxidative stress [15], hypoxia [24], and nitric oxide [25]. Rivera et al. [26] reported that rats with genetically elevated angiotensin I-converting enzyme activity overexpressed PAI-1 and MCP-1 mRNAs in aortic walls, and that treatment with fasudil, another inhibitor of ROCK, reduced their expression levels. In the present study, we showed that RhoA induced PAI-1 and MCP-1 expression in 3T3-L1 adipocytes through ROCK (Fig. 2D). Thus, similar to aortic walls, the RhoA-ROCK pathway seems to induce PAI-1 and MCP-1 in adipocytes.

TNF-α activates RhoA through tumor necrosis factor receptor 1 (TNF-R1), and subsequent activation of RhoA is required for TNF-α-induced phosphorylation of myosin phosphatase in airway smooth muscle cells [27,28]. Inhibition of ROCK prevents TNF-α-induced stress fiber formation in endothelial cells [29]. Recently, Mong et al. [30] reported that Y-27632 prevented TNF-α-induced JNK activation in pulmonary endothelial cells. In agreement with these reports, Y-27632 inhibited TNF-α-induced expression of inflammatory cytokines, such as MCP-1 and PAI-1 in this study (Fig. 3B). These results suggest that ROCK can modulate the signaling pathway of TNF-α.

In the present study, high RhoA activity was noted in adipose tissues of C57BL/6J mice, and fractionation study signified high levels of phosphorylated ERM in mature adipocyte fraction, suggesting that mature adipocytes contribute to the total RhoA activity in adipose tissues. In this regard, RhoA activation is reported to be relatively low in 3T3-L1 adipocytes [12,31]. Here, we showed, for the first time, that TNF-α and dexamethasone activated RhoA in 3T3-L1 adipocytes (Fig. 3A). Moreover, previous reports showed

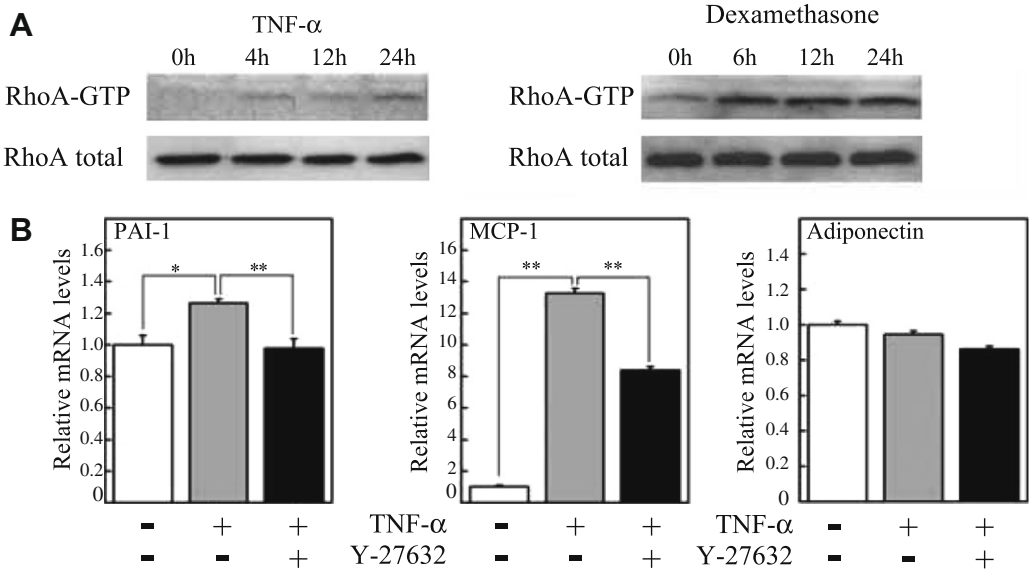

Fig. 3. Effects of TNF-α on RhoA activity and mRNA expression levels of inflammatory cytokines in 3T3-L1 adipocytes. (A) Cell lysates were collected from 3T3-L1 adipocytes cells treated with 10 ng/ml TNF-α (left panel) or 20 nM dexamethasone (right panel) for the indicated duration. Then, 50 μg of each cell lysates were subjected to SDS–PAGE and Western blotting with anti-RhoA (RhoA total). RhoA-GTP was isolated using GST-Rhotekin RBD, followed by SDS–PAGE and Western blotting with anti-RhoA Ab. (B) 3T3-L1 adipocytes were pretreated with or without 30 μM Y-27632 for 20 h, followed by treatment with or without 10 ng/ml TNF-α for 4 h. The mRNA levels were quantified by real-time RT-PCR. Values are normalized to the level of cyclophilin mRNA and expressed as mean ± SEM (n = 4–6).

that RhoA was activated by angiotensin II [32] in cardiac myocytes and by IL-1β or H_2O_2 in epithelial cells [33]. Taken together, these circulating factors may activate RhoA in adipose cells *in vivo*.

In summary, the results of the present study suggest that RhoA-ROCK pathway is one of the inducers of inflammatory cytokines in adipocytes.

Acknowledgments

The authors thank Yoshimi Takai (Kobe University, Japan) for the helpful discussion. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, Japan Heart Foundation/Pfizer Grant for Research on Hypertension, Hyperlipidemia and Vascular Metabolism, and AstraZeneca Research Grant.

References

[1] H. Xu, G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, J. Sole, A. Nichols, J.S. Ross, L.A. Tartaglia, H. Chen, Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J. Clin. Invest. 112 (2003) 1821–1830.
[2] P. Trayhurn, Endocrine and signalling role of adipose tissue: new perspectives on fat, Acta Physiol. Scand. 184 (2005) 285–293.
[3] Y. Matsuzawa, The metabolic syndrome and adipocytokines, FEBS Lett. 580 (2006) 2917–2921.
[4] G.S. Hotamisligil, N.S. Shargill, B.M. Spiegelman, Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance, Science 259 (1993) 87–91.
[5] A.H. Berg, P.E. Scherer, Adipose tissue inflammation, and cardiovascular disease, Circ. Res. 96 (2005) 939–949.
[6] H.R. Lijnen, Elements of the fibrinolytic system, Ann. NY Acad. Sci. 936 (2001) 226–236.
[7] A. Mavri, M. Stegnar, M. Krebs, J.T. Sentocnik, M. Geiger, B.R. Binder, Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women, Arterioscler. Thromb. Vasc. Biol. 19 (1999) 1582–1587.
[8] B.M. De Taeye, T. Novitskaya, L. Gleaves, J.W. Covington, D.E. Vaughan, Bone marrow plasminogen activator inhibitor-1 influences the development of obesity, J. Biol. Chem. 281 (2006) 32796–32805.
[9] B.J. Rollins, Monocyte chemoattractant protein 1: a potential regulator of monocyte recruitment in inflammatory disease, Mol. Med. Today 2 (1996) 198–204.
[10] H. Kanda, S. Tateya, Y. Tamori, K. Kotani, K. Hiasa, R. Kitazawa, S. Kitazawa, H. Miyachi, S. Maeda, K. Egashira, M. Kasuga, MCP-1 contributes to macrophage infiltration into adipose tissue insulin resistance, and hepatic steatosis in obesity, J. Clin. Invest. 116 (2006) 1494–1505.
[11] Y. Takai, T. Sasaki, T. Matozaki, Small GTP-binding proteins, Physiol. Rev. 81 (2001) 153–208.
[12] R. Sordella, W. Jiang, G.C. Chen, M. Curto, J. Settleman, Modulation of Rho GTPase signaling regulates a switch between adipogenesis and myogenesis, Cell 113 (2003) 147–158.
[13] M. Iwaki, M. Matsuda, N. Maeda, T. Funahashi, Y. Matsuzawa, M. Makishima, I. Shimomura, Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors, Diabetes 52 (2003) 1655–1663.
[14] X.D. Ren, W.B. Kiosses, M.A. Schwartz, Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton, EMBO J. 18 (1999) 578–585.
[15] S. Furukawa, T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda, I. Shimomura, Increased oxidative stress in obesity and its impact on metabolic syndrome, J. Clin. Invest. 114 (2004) 1752–1761.
[16] T. Matsui, M. Maeda, Y. Doi, S. Yonemura, M. Amano, K. Kaibuchi, S. Tsukita, Rho-kinase phosphorylates COOH-terminal threonines of ezrin/radixin/moesin (ERM) proteins and regulates their head-to-tail association, J. Cell Biol. 140 (1998) 647–657.
[17] L.A. Banaszynski, L.C. Chen, L.A. Maynard-Smith, A.G. Ooi, T.J. Wandless, A rapid reversible and tunable method to regulate protein function in living cells using synthetic small molecules, Cell 126 (2006) 995–1004.
[18] L.A. Maynard-Smith, L.C. Chen, L.A. Banaszynski, A.G. Ooi, T.J. Wandless, A directed approach for engineering conditional protein stability using biologically silent small molecules, J. Biol. Chem. 282 (2007) 24866–24872.
[19] H. Neumann, R. Schweigreiter, T. Yamashita, K. Rosenkranz, H. Wekerle, Y.A. Barde, Tumor necrosis factor inhibits neurite outgrowth and branching of hippocampal neurons by a rho-dependent mechanism, J. Neurosci. 22 (2002) 854–862.
[20] R.F. Ransom, N.G. Lam, M.A. Hallett, S.J. Atkinson, W.E. Smoyer, Glucocorticoids protect and enhance recovery of cultured murine podocytes via actin filament stabilization, Kidney Int. 68 (2005) 2473–2483.
[21] N. Furukawa, P. Ongusaha, W.J. Jahng, K. Araki, C.S. Choi, H.J. Kim, Y.H. Lee, K. Kaibuchi, B.B. Kahn, H. Masuzaki, J.K. Kim, S.W. Lee, Y.B. Kim, Role of Rho-kinase in regulation of insulin action and glucose homeostasis, Cell Metab. 2 (2005) 119–129.
[22] M. Fasshauer, J. Klein, S. Kralisch, M. Klier, U. Lossner, M. Bluher, R. Paschke, Monocyte chemoattractant protein 1 expression is stimulated by growth hormone and interleukin-6 in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun. 317 (2004) 598–604.
[23] T. Sakamoto, J. Woodcock-Mitchell, K. Marutsuka, J.J. Mitchell, B.E. Sobel, S. Fujii, TNF-alpha Insulin alone synergistically induce plasminogen activator inhibitor-1 expression in adipocytes, Am. J. Physiol. 276 (1999) C1391–C1397.
[24] B. Chen, K.S. Lam, Y. Wang, D. Wu, M.C. Lam, J. Shen, L. Wong, R.L. Hoo, J. Zhang, A. Xu, Hypoxia dysregulates the production of adiponectin and plasminogen activator inhibitor-1 independent of reactive oxygen species in adipocytes, Biochem. Biophys. Res. Commun. 341 (2006) 549–556.
[25] M. Nozaki, A. Fukuhara, K. Segawa, Y. Okuno, M. Abe, N. Hosogai, M. Matsuda, R. Komuro, I. Shimomura, Nitric oxide dysregulates adipocytokine expression in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun. 364 (2007) 33–39.
[26] P. Rivera, M.P. Ocaranza, S. Lavandero, J.E. Jalil, Rho kinase activation and gene expression related to vascular remodeling in normotensive rats with high angiotensin I converting enzyme levels, Hypertension 50 (2007) 792–798.
[27] I. Hunter, H.J. Cobban, P. Vandenabeele, D.J. MacEwan, G.F. Nixon, Tumor necrosis factor-alpha-induced activation of RhoA in airway smooth muscle cells: role in the Ca2+ sensitization of myosin light chain20 phosphorylation, Mol. Pharmacol. 63 (2003) 714–721.
[28] I. Hunter, G.F. Nixon, Spatial compartmentalization of tumor necrosis factor (TNF) receptor 1-dependent signaling pathways in human airway smooth muscle cells. Lipid rafts are essential for TNF-alpha-mediated activation of RhoA but dispensable for the activation of the NF-kappaB MAPK pathways, J. Biol. Chem. 281 (2006) 34705–34715.
[29] I. Petrache, A.D. Verin, M.T. Crow, A. Birukova, F. Liu, J.G. Garcia, Differential effect of MLC kinase in TNF-alpha-induced endothelial cell apoptosis and barrier dysfunction, Am. J. Physiol. Lung Cell. Mol. Physiol. 280 (2001) L1168–L1178.
[30] P.Y. Mong, C. Petrulio, H.L. Kaufman, Q. Wang, Activation of Rho kinase by TNF-alpha is required for JNK activation in human pulmonary microvascular endothelial cells, J. Immunol. 180 (2008) 550–558.
[31] R. McBeath, D.M. Pirone, C.M. Nelson, K. Bhadriraju, C.S. Chen, Cell shape cytoskeletal tension and RhoA regulate stem cell lineage commitment, Dev. Cell 6 (2004) 483–495.
[32] H. Aoki, S. Izumo, J. Sadoshima, Angiotensin II activates RhoA in cardiac myocytes: a critical role of RhoA in angiotensin II-induced premyofibril formation, Circ. Res. 82 (1998) 666–676.
[33] M.L. Roberts, L.M. Cowsert, Interleukin-1 beta reactive oxygen species mediate activation of c-Jun NH2-terminal kinases in human epithelial cells by two independent pathways, Biochem. Biophys. Res. Commun. 251 (1998) 166–172.