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Phenylenediamine derivatives induce GDF-15/MIC-1 and inhibit adipocyte differentiation of mouse 3T3-L1 Cells

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ABSTRACT

Phenylenediamine derivatives can function as a hydrogen donor and reportedly exert various biological actions including cytoprotective effects against oxidative stress, possibly by acting as an antioxidant. Previous studies showed that feeding of such compounds to mice reduced their body weight, but the precise mechanism remains unknown at present. Here, we found that these compounds inhibited the *in vitro* differentiation of mouse preadipocytes, 3T3-L1 cells, into adipocytes, suggesting that, at least in part, reduced generation of adipocytes might contribute to the observed weight loss in mice. Next, we performed array analysis and found that the expression of GDF-15/MIC-1, which is a TGFβ superfamily cytokine, and Trib 3, an intracellular downstream effector of the cytokines, was up-regulated by these derivatives. Thus, we identified the compounds as inducers of GDF-15/MIC-1 and suggest that such induction may have led to inhibition of adipocyte differentiation, which could account for the weight-loss effect of these compounds.

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1. Introduction

Obesity arises from an imbalance in energy intake and energy expenditure that leads to the pathological growth of adipocytes [1,2]. Obesity is characterized at the cellular level by an increase in the number and size of adipocytes differentiated from fibroblastic preadipocytes in adipose tissue [1,2], and it is induced by the hypertrophy of adipocytes and the generation of new adipocytes from precursor cells [3,4]. This differentiation is considered to consist of 2 distinctive steps, i.e., determination (fibroblasts to preadipocytes) and commitment (preadipocytes to adipocytes) [3,4]. Many reports suggest that mouse 3T3-L1 cells are one of the most well-characterized and reliable *in vitro* models for studying the commitment of preadipocytes to differentiation into adipocytes [4–6]. Recently, GDF-15, also called MIC-1, NAG-1, PDF, and PLAB, which is a TGF-β superfamily cytokine, was identified as an adipokine secreted from human adipocytes and mouse 3T3-L1 [9]. GDF-15 expression is associated with cellular stress or apoptosis, and further investigation of this cytokine has been focused on its involvement in ineffective erythropoiesis [9–11]. Remarkably high serum levels of it are detected in patients with thalassemia syndromes, congenital dyserythropoiesis, and some acquired sideroblastic anemias [10,11]. Further, GDF-15 has been identified as a main factor of weight loss due to appetite suppression, suggesting the proposition that inducers of GDF-15 might be used as therapeutic agents against obesity [10].

Phenylenediamine derivatives such as *N*,*N*′-diphenyl-*p*-phenylenediamine (DPPD) exert cytoprotective effects *in vitro* against oxidative stress in various types of cells including cortical neurons in primary culture and neuronal cell lines PC12 and HT22 [12–14]. DPPD supposedly protects neuronal cells against oxidative stress at least in part by acting as an antioxidant [13]. The long-term feeding of DPPD to rats [15] results in a dose-dependent reduction in body weight, and one possible mechanism may involve reduced generation of adipocytes [15]. Thus, we examined the effects of phenylenediamine derivatives on adipocyte differentiation *in vitro* and found that they were indeed inhibitory. As there are no reports on investigations into the molecular mechanisms underlying the effects that these compounds exert on adipogenesis, this present study is the first to demonstrate that phenylenediamine derivatives inhibit adipocyte differentiation.

Abbreviations: AhR, arylhydrocarbon receptor; ARE, antioxidant-response element; DAPI, 4′,6-diamino-2-phenylindole; DMSO, dimethyl sulfoxide; DMEM, Dulbecco's modified Eagle's medium; DPPD, *N*,*N*′-diphenyl-*p*-phenylenediamine; FBS, fetal bovine serum; IBMX, isobutyl-3-methylxanthine; PADA, *N*-phenyl-*p*-phenylenediamine; PBS, Ca^{2+}, Mg^{2+} (−)-phosphate-buffered saline; RT-PCR, reverse transcription polymerase chain reaction.

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2. Materials and methods

2.1. Chemicals

Phenylenediamine derivatives shown in Table 1 were obtained from Ouchi Shinko Chemical Industrial (Tokyo, Japan). D10, D11, D12, and D13 were prepared by us for the present research. *N*-Methyl- and *N*,*N*-dimethyl-*N*′-phenyl-*p*-phenylenediamine (D10 and D11, respectively) were prepared by reductive alkylation of *N*-phenyl-*p*-phenylenediamine (PADA) and paraformaldehyde and subsequent chromatographic separation. On the other hand, *N*-ethyl- and *N*,*N*-diethyl-derivative (D12 and D13, respectively) were prepared by direct alkylation of PADA with ethyl bromide. The compounds were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the culture medium was 0.1%. Isobutyl-3-methylxanthine (IBMX), dexamethasone, insulin, piloglitazone and Oil red O were obtained from Sigma–Aldrich Chemical Co. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), bovine calf serum, and penicillin–streptomycin came from Life Technologies from Invitrogen (Carsbad, CA).

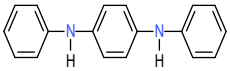
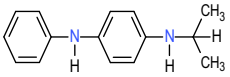
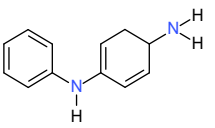
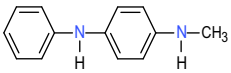
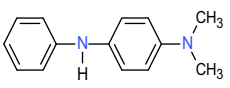
2.2. Cell culture

Mouse 3T3-L1 fibroblasts were maintained in DMEM containing 25 mM glucose supplemented with 10% calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine at 37 °C in a humidified atmosphere of 5% CO_2 [7,16]. The cells were plated in 6-well plates at a density that allowed them to reach confluence in 3 days. At this point (day 0), the medium was switched to differentiation medium (DMEM, 10% FBS, 0.25 μM dexamethasone, 0.5 mM IBMX, and 10 μg/ml insulin, and 1 μM piloglitazone); and these cells were kept in it for 2 days. Then various concentrations of the test compounds were added to the differentiation medium. On day 3 after their addition, the medium was replaced with that lacking dexamethasone and IBMX but leaving insulin in the cell medium for an additional 2 days. Thereafter, the cells were maintained in the original propagation DMEM with medium changes every 2 days [7,16].

2.3. Oil red O and DAPI staining

The cells were washed with ice-cold PBS(−) twice, fixed with 4% formalin at room temperature for 1 h, and stained with 0.2% Oil red O in isopropanol for 10 min [7,16]. Thereafter they were stained with DPAI (5 μM) in PBS(−) and then washed with PBS(−). Images were obtained by use of an Olympus (Tokyo, Japan) microscope [7,16].

Table 1
Chemical structures of the phenylenediamine derivatives and effective dose of 50% inhibition (IC_{50}) of adipocyte differentiation.

Abbreviation	Chemical structures	IC_{50} [μM]
DPPD		0.97
D1		0.67
D2		9.55
D10		6.48
D11		3.99

2.4. Oligonucleotide microarray analysis

Total RNA was isolated by TRIzol Reagent (Invitrogen, Carlsbad, CA) from vehicle- or D11- (10 μM) treated 3T3-L1 cells [16–18]. cDNA was synthesized by using the Superscript II system (Invitrogen) with a T7-Oligo(dT) primer. Biotin-labeled cRNA was prepared by *in vitro* transcription and fragmented by incubation at 94 °C for 35 min in 40 mmol/L Tris acetate buffer (pH 8.1) containing 100 mmol/L potassium and 30 mmol/L magnesium acetate. Fragmented cRNA was hybridized at 45 °C for 16 h to a GeneChip® Mouse 430 2.0 Array (Affymetrix, Santa Clara, CA), which contained over 39000 transcripts. Probe Arrays were washed and stained by using a Fluidics Station 450 and scanned with a GeneChip® Scanner 3000. Affymetrix GeneChip Operating Software (GCOS v1.4) was used for the analysis. To increase the reliability of the data, we listed the genes that had detection *p*-values of <0.001 with respect to the control (vehicle-treated cells).

2.5. RT-PCR

For reverse transcription-polymerase chain reaction (RT-PCR) analysis, total RNA of 3T3-L1 cells was obtained at 24.0 h after D11 (10 μM) treatment by use of TRIzol Reagent (Invitrogen) [15–17]. cDNA was synthesized by using the Superscript III system (Invitrogen). One one-hundredth of the cDNA was used for 1 PCR reaction. At the completion of the PCR, 10 μl of PCR products was mixed with 2 μl of loading buffer and electrophoresed in 1.5% agarose gel in the presence of 0.5 μg/ml of ethidium bromide. The following primers were used to amplify the cDNA fragments, which were visualized with a UV transilluminator: β-actin (287 bp) F: 5′-ATC CGT AAA GAC CTC TAT GC-3′, R: 5′-AAC GCA GCT CAG TAA CAG TC-3′; Gdf15 (257 bp) F: 5′-CGC CCT GGC AAT GCC TGA AC-3′, R: 5′-GCA CGC GGT AGG CTT CGG GG-3′; Trib3 (63 bp) F: 5′-CTG AGG CTC CAG GAC AAG A-3′, R: 5′-CCT GCA GGA AAC ATC AGC A-3′.

2.6. Statistical analysis

Results were presented as means ± standard deviation (SD). Data were analyzed by using SAS software. Analysis of variance was performed by use of *t*-test procedures.

3. Results

3.1. Effect of phenylenediamine derivatives on adipocyte differentiation

In the present study, we first compared the inhibition of adipocyte differentiation by these compounds. To observe the effects of the compounds on adipocyte differentiation, we caused the 3T3-L1 cells to differentiate in differentiation medium containing D11 or not (Fig. 1A) [7,16]. For visualization of lipid accumulation and nuclei, the cells were stained with Oil red O and DAPI, respectively (Fig. 1A). The cells treated with vehicle in the normal medium did not shown any accumulation of lipids (data not shown). In contrast, the cells incubated in the differentiation medium significantly produced lipid droplets (Fig. 2A), with 70% of the total cells having accumulated lipid droplets. D11 did not show any toxic effects on 3T3-L1 cells at least up to 10 μM (data not shown). However, it significantly reduced the percentage of lipid-accumu-

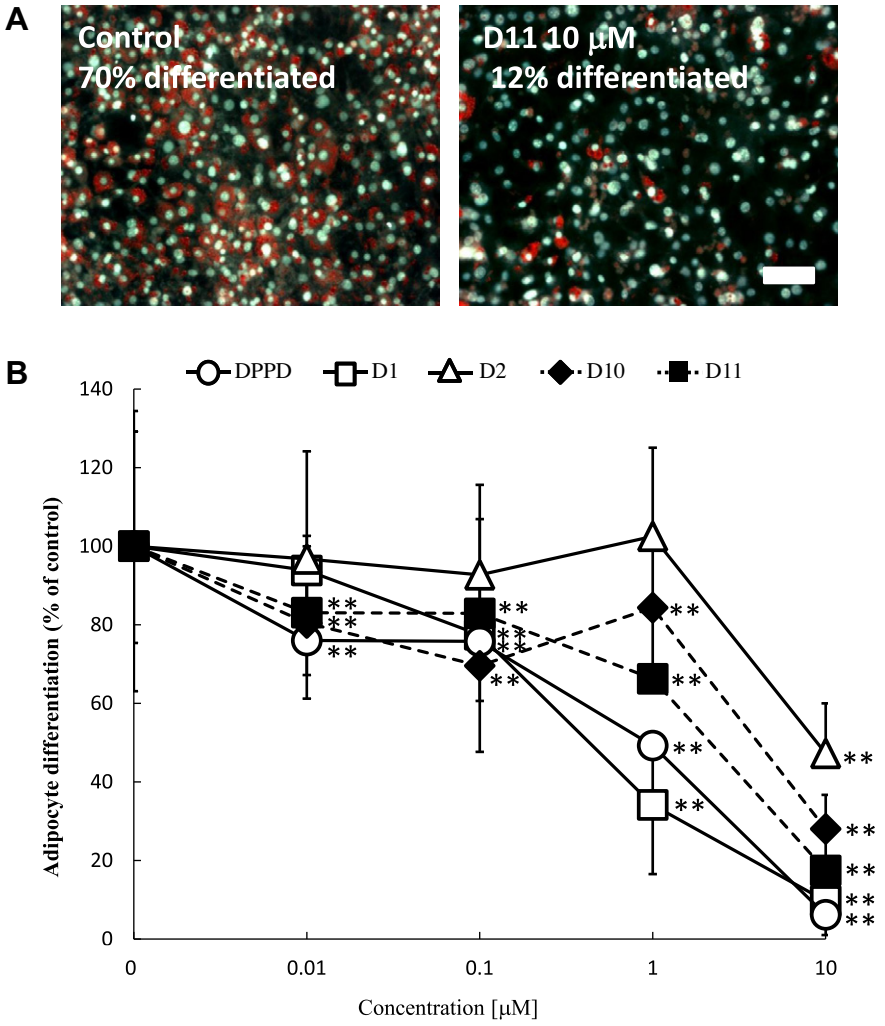


Fig. 1. (A) D11 inhibited adipocyte differentiation. 3T3-L1 cells were cultured in differentiation medium in the presence of vehicle (DMSO) as the control or D11 (10 μM). Then the cells were fixed and stained with Oil red O (red) and DAPI (blue). The scale bar indicates 10 μm. (B) Dose–response curve of the phenylenediamine derivatives. 3T3-L1 cells were incubated in the presence of various concentrations of the indicated compounds. In order to quantify the inhibitory effects of the compounds, we set the percentage of lipid-accumulating cells (control cells) as 100% and compared the effect of each compound with this value. ** indicate significant differences from the control ($P < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lating cells to only 12% (Fig. 1A). The compounds tested here potently and dose-dependently decreased the number of cells that accumulated lipid in their cytoplasm (Fig. 1B).

3.2. DNA microarray analysis and RT-PCR

Next, we performed array analysis using D11 in order to identify the gene(s) responsible for the neuroprotective effect afforded by these compounds (Table 2). The cells were incubated with D11 (10 μM) or vehicle, and total RNA was isolated and subjected to reverse-transcription and microarray analysis using the synthesized cDNA. DPPD was earlier reported to activate arylhydrocarbon receptors [19], but D11 did not induce phase-1 genes. Thus, D11 may be safer than DPPD. In contrast, D11 induced phase-2 genes (Chac1, Slc7a1, Sld6ab and Slc7a5), suggesting that D11 might have activated the Nrf2/ARE pathway [20–23]. The top 20 genes induced by D11 (10 μM) are listed in Table 2. The genes most strongly induced were GDF-15 and Trib 3, both of which are involved in the same signaling pathway [9–11]. In order to detect the induction of the GDF-15 and Trib 3 genes, we performed RT-PCR using total RNA from 3T3-L1 cells pretreated with 10 μM D1 (Fig. 2A). The β-actin gene was used as an internal positive control, and its expression level was not changed by D11. The expression of both of the above genes was increased by D11 after 24 h of incubation, although the patterns of induction were slightly different between them. Next, we examined whether GDF-15/MIC-1 itself could inhibit the differentiation of into adipocytes (Fig. 2B). But GDF-15/MIC-1 itself did not inhibit the differentiation, suggesting that coordinated induction of genes or induction of some other gene was required.

4. Discussion

4.1. Phenylenediamine derivatives inhibit adipocyte differentiation

Why does the long-tem feeding of *phenylenediamine derivatives* induce weight loss? Recent reports indicate that one of the critical events for the accumulation of body fat is the differentiation of preadipocytes into adipocyte in the adipose tissue [3–6]. Thus we suspected that the inhibition of such differentiation might contribute to the anti-obesity action *in vivo*, and so we planned the present experiments using 3T3-L1 cells [7,8]. Our results indicated that the compounds inhibited adipocyte differentiation at very low concentrations (D11, IC_{50} = 3.99 μM). In light of this present data the

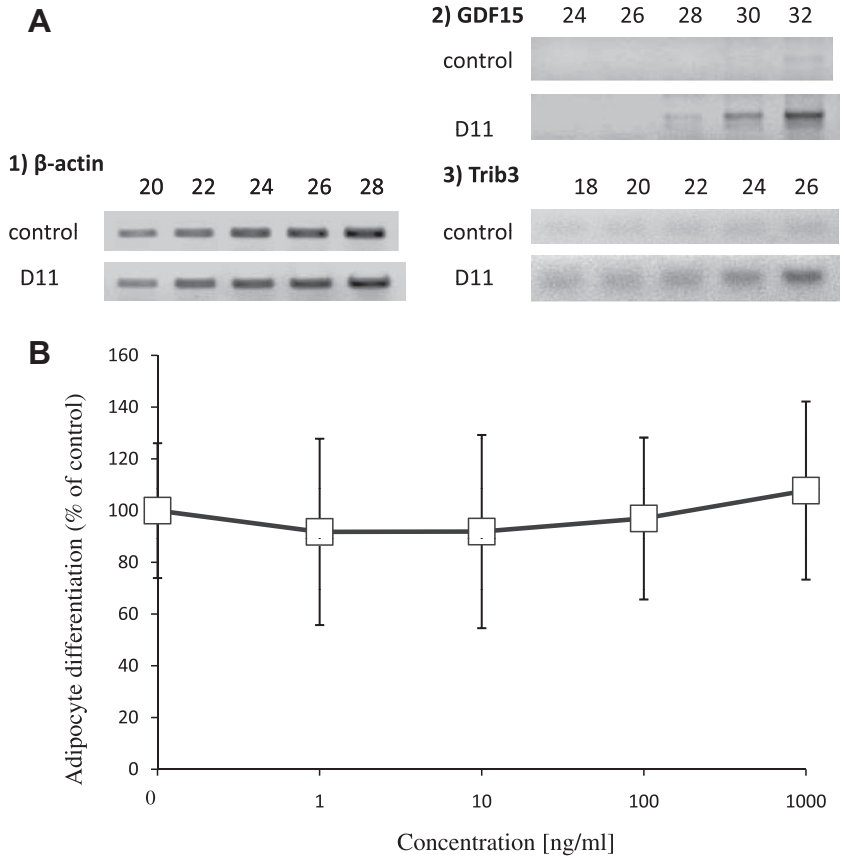


Fig. 2. (A) Induction of GDF-15 and Trib 3 genes. Total RNAs were extracted from the 3T3-L1cells that had been treated with 5 μM CA for 24 h. RT-PCR was performed by using the templates of cDNA at the indicated cycles. (B) Dose–response curve of GDF-15. 3T3-L1 cells were incubated in the presence of various concentrations of GDF-15. In order to quantify the inhibitory effects of GDF-15, we set the percentage of lipid-accumulating cells (control cells) as 100% and compared the effect of each compound with this value. Note that there were no significant differences at any concentration.

Table 2
Top 20 genes induced by D11 in 3T3-L1 cells.

No.	Induction	GeneBank	Gene description	Gene symbol
1	29.9	NM_011819	Growth differentiation factor 15	Gdf15
2	21.4	BB508622	Tribbles homolog 3 (Drosophila)	Trib3
3	12.3	BC012955	Tribbles homolog 3 (Drosophila)	Trib3
4	8.4	M94967	Prostaglandin-endoperoxide synthase 2	Ptgs2
5	8.3	BC025169	ChaC, cation transport regulator-like 1 (E. coli)	Chac1
6	6.1	BB264620	Solute carrier family 7 (cationic amino acid transporter, *y* + system), member 1	Slc7a1
7	5.6	BC019946	Activating transcription factor 3	Atf3
8	5.6	NM_010591	Jun oncogene	Jun
9	5.5	NM_134038	Solute carrier family 16 (monocarboxylic acid transporters), member 6	Slc16a6
10	5.5	NM_011799	Cell division cycle 6 homolog (*S. cerevisiae*)	Cdc6
11	4.8	AF477481	Chromatin licensing and DNA replication factor 1	Cdt1
12	4.7	BE629588	Zinc finger protein 367	Zfp367
13	4.7	BC011440	H2b histone family member /// predicted gene, OTTMUSG00000013203	LOC665622 /// RP23–38E20.1
14	4.6	BC026131	Similar to solute carrier family 7 (cationic amino acid transporter, *y* + system), member 5 /// solute carrier family 7 (cationic amino acid transporter, y + system), member 5	LOC100047619 /// Slc7a5
15	4.5	BB264620	Solute carrier family 7 (cationic amino acid transporter, *y* + system), member 1	Slc7a1
16	4.4	NM_008350	Interleukin 11	Il11
17	4.4	AF477481	Chromatin licensing and DNA replication factor 1	Cdt1
18	4.3	BC004617	Transcription factor 19	Tcf19
19	4.2	NM_021274	Chemokine (C-X-C motif) ligand 10	Cxcl10
20	4.1	NM_008102	GTP cyclohydrolase 1	Gch1

These genes were selected for their significant up-regulation ($p < 0.0003$) in response to D11. 3T3-L1 cells were incubated in the absence or presence of 10 μM D11 or vehicle for 24 h in normal medium. Total RNA was isolated and subjected to microarray analysis. Fold change (D11/vehicle), Public ID, gene descriptions, and gene symbols are indicated.

compounds could thus have exerted their anti-obesity action by inhibiting adipocyte differentiation. We consider that there are three possible pathways leading to inhibition of adipocyte differentiation: (1) antioxidant activities [12–14], (2) induction of phase 2 enzymes by activation of the Nrf2/ARE pathway [20–23], and (3) activation of GDF-15/Trib three pathways [9–11].

4.2. Phenylenediamine derivatives as GDF-15 inducers without toxic effects

Because all known GDF-15 inducers such as hydrogen peroxide and prostaglandin J_2 also cause severe damage to cells [9], a GDF-15 inducer without toxic effects is required [9,10]. Based on our results presented here, we suggest that pheneylenediamine derivatives may be such GDF-15 inducers. As was shown by array analysis (Table 2), GDF-15 induction was prominent, and so one possible mechanism for the inhibition of adipocyte differentiation might be the activation of the GDF-15/Trib 3 pathway. Because GDF-15 alone did not inhibit the differentiation (Fig. 2B), the coordinated activation of the pathway, for example, the induction of Trib 3 protein, might be required for the inhibition. Both proteins are involved in the TGFβ superfamily signaling, and both of them are reported to participate in the regulation of obesity [9–11]. First, GDF-15/MIC-1 is known as a main factor of anorexia (appetite loss) during the late phase in cancer patients [10]. GDF-15/MIC-1 is a secretory product of both preadipocytes and differentiated adipocytes [9]. Recombinant GDF-15/MIC-1 enhances adiponectin release from adipocytes, suggesting that GDF-15/MIC-1 is a positive regulator of adiponectin [9]. In addition, GDF-15/MIC-1 expression in human adipose tissue is negatively associated with body fat mass and may influence adiposity [9]. Finally, GDF-15/MIC-1 is a novel adipokine that may well have a paracrine role in the modulation of adipose tissue function and could even be a potential target for obesity treatment [9]. Further, Trib 3, which is known as an intracellular effector of TGFβ family cytokines [11], can inhibit lipid accumulation and can be induced by phenamil, another diphenylenediamine-like compound [24]. As phenamil induces osteoblast differentiation by activating Trib 3 [24], the diphenylenediamine derivatives might have inhibited adipocyte differentiation by such activation. Thus, the coordinated activation of the GDF-15 and Trib 3 pathway by phenylenediamine derivatives might be essential for the weight-loss effect noted by long-term feeding of DPPD [15].

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References

[1] T.L. Visscher, J.C. Seidell, The public health impact of obesity, Annu. Rev. Public Health 22 (2001) 355–375.
[2] P.G. Kopelman, Obesity as a medical problem, Nature 404 (2000) 635–643.
[3] M.D. Lane, Q.Q. Tang, From multipotent stem cell to adipocyte, Birth. Defects. Res. A Clin. Mol. Terat. 73 (2005) 476–477.
[4] R.M. Cowherd, R.E. Lyle, R.E. Mcgehee Jr., Molecular regulation of adipocytedifferentiation, Semin. Cell Dev. Biol. 10 (1999) 2–10.
[5] S. Rayalam, M.A. Della-Fera, C.F. Baile, Phytochemicals and regulation of the adipocyte life cycle, J. Nutr. Biochem. 19 (2008) 717–726.
[6] C.L. Hsu, G.C. Yen, Phenolic compounds: evidence for inhibitory effects against obesity and their underlying molecular signaling mechanism, Mol. Nutr. Food Res. 52 (2008) 53–61.
[7] T. Kawada, N. Aoki, Y. Kamei, K. Maeshige, S. Nishiu, E. Sugimoto, Comparative investigation of vitamins and their analogues on terminal differentiation, from preadipocytes to adipocytes, of 3T3-L1, Comp. Biochem. Physiol., A: Comp. Physiol. 96 (1990) 323–326.
[8] 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.
[9] Q. Ding, T. Mracek, P. Gonzalez-Muniesa, K. Kos, J. Wilding, P. Trayhurn, C. Bing, Identification of macrophage inhibitory cytokine-1 in adipose tissue and its secretion as an adipokine by human adipocytes, Endocrinology 150 (2009) 1688–1696.
[10] H. Johnen, S. Lin, T. Kuffner, D.A. Brown, V.W. Tsai, A.R. Bauskin, L. Wu, G. Pankhurst, L. Jiang, S. Junankar, M. Hunter, W.D. Fairlie, N.J. Lee, R.F. Enriquez, P.A. Baldock, E. Corey, F.S. Apple, M.M. Murakami, E.J. Lin, C. Wang, M.J. During, A. Sainsbury, H. Herzog, S.N. Breit, Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1, Nat. Med. 13 (2007) 1333–1340.
[11] L. Qi, J.E. Heredia, J.Y. Altarejos, R. Screaton, N. Goebel, S. Niessen, I.X. Macleod, C.W. Liew, R.N. Kulkarni, J. Bain, C. Newgard, M. Nelson, R.M. Evans, J. Yates, M. Montminy, TRB3 links the E3 ubiquitin ligase COP1 to lipid metabolism, Science 312 (2006) 1763–1766.
[12] T. Satoh, M. Izumi, Neuroprotective effects of phenylenediamine derivatives independent of an antioxidant pathway in neuronal HT22 cells, Neurosci. Lett. 418 (2007) 102–105.
[13] T. Satoh, N. Sakai, Y. Enokido, Y. Uchiyama, H. Hatanaka, Survival factor-insensitive generation of reactive oxygen species induced by serum deprivation in neuronal cells, Brain Res. 739 (1996) 9–14.
[14] H. Aoshima, T. Satoh, N. Sakai, M. Yamada, Y. Enokido, T. Ikeuchi, H. Hatanaka, Generation of free radicals during lipid hydroperoxide-triggered apoptosis in PC12 cells, Biophys. Biochem. Acta. 1345 (1997) 35–42.
[15] R. Hasegawa, S. Fukushima, A. Hagiwara, T. Masui, A. Masuda, N. Ito, Long-term feeding study of *N*,*N*′-diphenyl-*p*-phenylenediamine in F344 rats, Toxicology 54 (1989) 69–78.
[16] T. Takahashi, T. Tabuchi, Y. Tamaki, K. Kosaka, Y. Takikawa, T. Satoh, Carnosic acid and carnosol inhibit adipocyte differentiation in mouse 3T3-L1 cells through induction of phase 2 enzymes and activation of glutathione metabolism, Biochem. Biophys. Res. Commun. 382 (2009) 549–554.
[17] Y. Tamaki, T. Tabuchi, T. Takahashi, K. Kosaka, T. Satoh, Activated glutathione metabolism participates in protective effects of carnosic acid against oxidative stress in neuronal HT22 cells, Planta Med. 76 (2000) 683–688.
[18] S. Sasaki, T. Tozawa, R.M. Van Wagoner, C.M. Ireland, M.K. Harper, T. Satoh, Strongylophorine-8, a pro-electrophilic compound from the marine sponge Petrosia(Strongylophora) corticata, provides neuroprotection through Nrf2/ARE pathway, Biochem. Biophys. Res. Commun. 415 (2011) 6–10.
[19] K. Sugihara, T. Okayama, S. Kitamura, K. Yamashita, M. Yasuda, S. Miyairi, S. Ohta, Comparative study of aryl hydrocarbon receptor ligand activities of six chemicals in vitro and vivo, Arch. Toxicol. 82 (2008) 5–11.
[20] T. Satoh, T. Raraie, M. Seki, C.R. Sunico, T. Tabuchi, T. Kitagawa, M. Yanagitai, M. Senzaki, C. Kosegawa, H. Taira, S.R. Mckercher, J.K. Hoffman, G.P. Roth, S.A. Lipton, Dual neuroprotective pathways of a pro-electrophilic compound via HSF-1-activated heat-shock proteins and Nrf2-activated phase 2 antioxidant response enzymes, J. Neurochem. 119 (2011) 569–578.
[21] T. Satoh, S.A. Lipton, Redox regulation of neuronal survival by electrophilic compounds, Trends Neurosci. 30 (2007) 38–45.
[22] T. Satoh, K. Kosaka, K. Itoh, A. Kobayashi, M. Yamamoto, Y. Shimojo, C. Kitajima, J.Cui.J. Kamins, S. Okamoto, T. Shirasawa, S.A. Lipton, Carnosic acid, a *catechol-type* electrophilic compound, protects neurons both *in vitro* and *in vivo* through activation of the Keap1/Nrf2 pathway via S-alkylation of specific cysteines, J. Neurochem. 104 (2008) 1131–1161.
[23] T. Satoh, S. Okamoto, J. Cui, Y. Watanabe, K. Furuta, M. Suzuki, K. Tohyama, S.A. Lipton, Activation of the Keap1/Nrf2 pathway for neuroprotection by electrophilic phase II inducers, Proc. Natl Acad. Sci. USA 103 (2006) 768–773.
[24] K.W. Park, H. Waki, W.K. Kim, B.S. Davies, S.G. Young, F. Parhami, P. Tontonoz, The small molecule phenamil induces osteoblast differentiation and mineralization, Mol. Cell. Biol. 29 (2009) 3905–3914.