Food Sci. Biotechnol. Vol. 17, No. 2, pp. 267 ~ 273 (2008)

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Polysaccharides from Edible Mushroom *Hinmogi* (*Tremella fuciformis*) Inhibit Differentiation of 3T3-L1 Adipocytes by Reducing mRNA Expression of PPARγ, C/EBPα, and Leptin

Hye-Jin Jeong, Seon-Joo Yoon[1,2]*, and Yu-Ryang Pyun

Department of Biotechnology, Yonsei University, Seoul 120-749, Korea

[1]*Graduate Program in Biomaterials Science and Engineering, Yonsei University, Seoul 120-749, Korea*

[2]*Division of Biomembrane Research, Pacific Northwest Research Institute, University of Washington, Seattle, WA 98122, USA*

Abstract Water-soluble fraction (WSF) from edible mushroom *hinmogi* (*Tremella fuciformis*) were obtained by water extraction, and polysaccharides in the WSF were separated by ethanol precipitation. The inhibitory effects of the polysaccharides on 3T3-L1 adipocyte differentiation were evaluated by the reduction of peroxisome proliferators-activated receptor γ (PPARγ) translation, triglyceride accumulation, Oil Red-O staining, and expression levels of PPARγ, CCAAT/enhancer binding protein α (C/EBPα), and leptin. The PPARγ translation in 3T3-L1 cells was inhibited by the treatment with polysaccharide precipitated by 80% ethanol (P80) which showed highest inhibitory activity among polysaccharides tested. In addition, treatment of P80 to 3T3-L1 cells significantly inhibited the triglyceride accumulation, Oil Red-O staining, and mRNA expression of PPARγ, C/EBPα, and leptin in a dose-dependent manner. Based upon these results, P80 from edible mushroom *hinmogi* shows the inhibitory activity on the differentiation of 3T3-L1 adipocytes. Therefore, it might be employed as a potential anti-obesity material.

Keywords: edible mushroom, *Tremella fuciformis*, polysaccharide, differentiation, anti-obesity

Introduction

The 3T3-L1 adipocytes can be maintained in a stable cell monolayer, which maintains cell viability and hormonal responsiveness for several days. In addition, 3T3-L1 cells allow the investigation of the stimuli and mechanism regulating differentiation of preadipocytes to adipocytes (1). Preadipocytes isolated from adipose tissues and cloned cells exhibit similar characteristics with fibroblasts in the stage of the preadipocytes. As differentiation proceeds, the expression of genes for lipids and proteins, and the accumulation of fat droplets are observed as characteristics of adipocytes (2). When the 3T3-L1 cells grow to confluence, they convert, with a certain frequency, into adipose cells (3). In humans, it has been clarified that fat cell (adipocytes, which comprises adipose tissue) differentiation and the extent of subsequent fat accumulation are closely related to the occurrence and advancement of various diseases such as obesity, type II diabetes, coronary artery disease, and hypertension (2). It was reported that adipocytes release many bioactive molecules including adipsin, angiotensionogen, leptin, tumor necrosis factor α (TNF-α), and adiponectin during their differentiation and maturation (4). Upon induction of adipocyte differentiation, a cascade of gene transcription events occur, leading to the expression of adipocyte-specific genes. The CCAAT-enhancer binding protein (C/EBP) family, a family of leucine zipper-type transcription factors, and the peroxisome proliferator-activated receptors (PPAR) family, a family of ligand-dependent receptor-type transcription factors, interact with each other, forming a network, which plays an important role as a master regulator. Leptin, a product of *ob* gene and a hormone primarily secreted from adipose tissue, reduces food intake and increases energy expenditure. The *aP2* is adipocyte specific gene regulated by PPARγ in 3T3-L1 (5).

Mushrooms valued as flavorful foods and medicinal substances for millennia and widely used as nutritional supplements. However, there has not been a critical review attempting to integrate their nutraceutical potential with basic science. Several mushrooms are claimed to exhibit biological actions, including antitumor and anti-inflammatory (6), anticoagulant (7), and anti-genotoxic activity (8). Recently we screened edible mushrooms, *hinmogi* (Korean name, *Tremella fuciformis*), with potent anti-obesity action. The body of the *hinmogi* has long been considered a common food, and is widely used as a traditional crude drug in China. In recent years, it has been found that the polysaccharide fraction composed of acidic and neutral heteroglycans from *hinmogi* has several pharmacological activities such as enhancing host immune functions (9,10), anti-tumor (11), hypoglycemic activity (12), and cytotoxic effects on human colon adenocarcinoma cell line DLD-1 (13). Prior to this report, however, systematic research on anti-obesity effects of the extract from *hinmogi* was not performed. In order to clarify and provide the scientific basis for anti-obesity action, we extracted and separated polysaccharides from *hinmogi*. Surprisingly, we obtained non-sulfated polysaccharides with potential anti-obesity activities through the inhibition of preadipocyte differentiation.

*Corresponding author: Tel: +1-206-860-6767; Fax: +1-206-726-1217
E-mail: yoonsj@u.washington.edu
Received June 12, 2007; accepted October 2, 2007

Materials and Methods

Materials *Hinmogi* (*Tremella fuciformis*) was purchased from a local market in China, and Mucosa material of porcine small intestine was from a local market in Korea. Dexamethasone, 3-isobutyl-1-methylxanthine, sucrose, and *p*-nitrophenyl-α-D-glucopyranoside were purchased from Sigma (St. Louis, MO, USA). Dulbecco's modified Eagle's Medium (DMEM) was from Irvine Scientific (Santa Ana, CA, USA). Fetal bovine serum (FBS), penicillin, streptomycin sulfate, and glutamine were purchased from Invitrogen (Carlsbad, CA, USA). Other reagents were from Sigma unless described otherwise.

Extraction and fractionation of polysaccharides from edible mushroom *hinmogi* Extraction and fractionation of polysaccharides were performed based on the previously described method (14). Briefly, the dried *hinmogi* was ground using a food mixer (model DS2200; Daesung Electronics, Seoul, Korea). The dried powder (10 g) of *hinmogi* was suspended in 1 L of 75% ethanol and extracted under reflux at 76°C for 1 hr. This procedure was repeated twice, and thereafter the suspension was then filtered with Whatman No. 2 paper (Maidstone, Kent, England) to remove the ethanol-soluble materials. The sediment was collected, suspended in 1 L of distilled water and refluxed for 2 hr at 97°C, and a clear supernatant was collected after centrifugation at 4,000×g for 20 min at 4°C. This extraction procedure was repeated twice and the supernatants obtained were pooled. The supernatant was concentrated in a rotary evaporator under reduced pressure. After filtration with Whatman No. 2 paper, the clear filtrate was lyophilized. This preparation is denominated as water-soluble fraction (WSF) from the *hinmogi*. After dissolving in distilled water, the WSF were precipitated with 20, 40, 60, and 80%(v/v) ethanol by keeping at 4°C for 24 hr. The resulting precipitates were filtered with Whatman No. 2 paper, washed with 80% ethanol, dried at room temperature, dissolved in water, and dialyzed against running water for 48 hr. The nondialyzed portion was centrifuged as described above to remove insoluble materials and the supernatant was lyophilized. Final products fractionated from WSF by ethanol with 20, 40, 60, and 80%(v/v) were denominated as P20, P40, P60, and P80, respectively.

Cell culture and differentiation induction of 3T3-L1 cells 3T3-L1 Preadipocytes were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The differentiation of the cells was induced by slightly modified method previously described (15). Briefly, 3T3-L1 preadipocytes were grown as monolayer cultures in 12-well culture plates at 37°C in a humidified atmosphere containing 5% CO_2 in DMEM containing 10% FBS, 50 units/mL penicillin, 50 μg/mL streptomycin sulfate, and 2 mM glutamine. The fresh medium was exchanged after every 48 hr. The differentiation of the cells grown to a confluence was induced by the addition of same volume of fresh DMEM containing 5 μg/mL insulin, 0.5 mM 3-isobutyl-1-methylxanthine, 0.75 μM dexamethasone, and either with or without the polysaccharides extracted from mushrooms. After induction for 48 hr, the differentiated cells were maintained in the same media without 3-isobutyl-1-methylxanthine and dexamethasone for an additional 2 days. The fresh medium was exchanged after every 2 days. The cells between 9 and 12 days after induction of differentiation were used for following experiments.

Cell lysate preparation Differentiated 3T3-L1 cells cultured in 12-well culture plates as described in above were washed with phosphate-buffered saline (PBS) and lysed in 1 mL of cold lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100) containing protease inhibitors and phosphates inhibitors (1 mM Na_3VO_4, 5 mM NaF, 10 mM *p*-nitrophenylphosphate, 10 mM β-glycerophosphate). The clear cell lysates were prepared and protein concentration was determined by BCA kit (Pierce, Rockford, IL, USA).

Triglyceride Oil Red-O staining and quantitative analysis Light microscopy and Oil Red-O staining were used to monitor the characteristic cell rounding and lipid accumulation during adipocyte differentiation as described previously (16). Briefly, cell monolayers in 12-well culture plates were washed with PBS, fixed with 10% neutral-buffered formalin for a 20 min, drained and covered with propylene glycol for 3 min. After removal of propylene glycol, the cell monolayers were covered with Oil Red-O solution. After 1 hr at room temperature, the Oil Red-O was removed, and the plates were washed twice with PBS. Triglycerides were visualized by light phase contrast microscopy. For quantitative determination, the cells in 12-well culture plates stained with Oil Red-O were subsequently destained with 60%(v/v) propylene glycol for 1 min under gentle agitation. The plates were exhaustively rinsed with water and dried in a 37°C dry oven for 1 hr. The dye in each dried culture plate was completely extracted by agitation in isopropanol. The extracted dye was immediately removed, brought to a constant volume with isopropanol, and the absorbance was measured at 510 nm. Total triglyceride accumulation in cells was measured after lipid extraction with the INT kit (Sigma) as described previously (17). Briefly, cells were maintained in 12-well culture plates for 2 days with or without polysaccharides extracted from mushroom, scraped in 3 mL of CH_3OH/H_2O (2 : 0.8, v/v), and triglycerides were extracted with 1 mL of $CHCl_3$. The aqueous phase was washed with 1 mL of $CHCl_3$, solvent was evaporated, and triglycerides were resuspended in 300 μL of $CHCl_3/CH_3OH$ (2 : 1, v/v) and quantified with the INT kit according to the manu-facturer's instructions.

Reverse-transcription polymerase chain reaction (RT-PCR) Total RNA from differentiated 3T3-L1 adipocytes was isolated with STAT-60 (Tel-Test Inc., Friendswood, TX, USA) as recommended by the manufacturer. Total RNA (1 μg) was used to produce cDNA using RT-PCR system. The reverse-transcription was performed at 42°C for 1 hr and heated at 95°C for 5 min to inactivate the Avian Myeloblastosis Virus Reverse Transcriptase (AMV RT) enzyme. The forward primer (5'-GGT GAA ACT CTG GGA GAT TC3') and reverse primer (5'-CAA CCA TTG GGT CAG CTC TT3') were used as cDNA primer of PPARγ, and forward primer (5'-AGG TGC TGG AGT TGA CCA GT3') and reverse primer (5'-CAG CCT AGA GAT CCA GCG AC3') were for C/EBPα. The reaction

were denatured at 94°C for 1 min, annealed at 54°C for 1 min and extended at 72°C for 30 sec. Amplification was carried out using 25 cycles, and final extension was performed at 72°C for 7 min. The forward primer (5'-CCA AAA CCC TCA TCA AGA CC3') and reverse primer (5'-CTC AAA GCC ACC ACC TCT GT3') were used as cDNA primer of leptin. To detect these transcription factors and *ob* gene, we inserted primers, forward (5'-CCA TCA ACG ACC CCT TCA3') and reverse 5'-GTC CTC AGT GTA GCC CAA GA3'), for housekeeping gene glyceralde-hydes-3-phosphate dehydrogenase (G3PDH) into each sample. The reactions were denatured at 94°C for 1 min, annealed at 57°C for 1 min, and extended at 72°C for 2 min. Amplification was carried out using 30 cycles and final extension was performed at 72°C for 7 min. The PCR products were loaded onto 1.5% agarose gel, stained with ethidium bromide and examined under UV.

Western blot analysis of PPARγ Western blot analysis of the cultured 3T3-L1 cells treated with or without mushroom extracts was carried out as described previously (18). Briefly, samples corresponding to 30 μg of protein were prepared in SDS/PAGE sample buffer containing 5% 2-mercaptoethanol and boiled for 5 min. Proteins were separated on 10%(w/v) acrylamide gels and transferred to PVDF membrane (Millipore, Billerica, MA, USA). The PVDF membrane was blocked with 5%(w/v) nonfat dry milk in TBS (140 mM $NaCl_2$, 10 mM Tris-HCl, pH 8.0) for 1 hr at room temperature, then washed 5 times with TBS and incubated for 1 hr with mouse monoclonal antibody that recognizes the C-terminus of PPARγ of human origin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a 1 : 1,000 dilution. The membrane was washed 5 times with TBS and incubated with an horseradish peroxide (HRP)-conjugated anti-mouse IgG antibody (SouthernBiotech, Birmingham, AL, USA) at a 1 : 3,000 dilution for 1 hr at room temperature. The membrane was washed 5 times with TBS and incubated with a Supersignal chemiluminescence substrate kit (Pierce, Rockford, IL, USA). For reprobing the same blots with anti-γ-tubulin antibody (Sigma), the blot was rinsed with distilled water briefly and then incubated with stripping buffer (2% SDS, 62.6 mM Tris-HCl, pH 6.7, 0.78% 2-mercaptoethanol) for 20 min at 60°C. After rinsing (5×5 min each) with TBS/0.05% Tween 20, the PVDF membrane was subjected to blocking procedure. All procedures after blocking were as described above. The intensity of bands was determined by densitometric analysis using a Scion imaging program.

Determination of molecular weight Ten mg of P80 obtained from *hinmogi* was dissolved in 3 mL of distilled water and applied to a gel filtration chromatography on Sephacry S-400 HR column (1.5×170 cm) equilibrated with 0.5 M ammonium bicarbonate (pH 7.0). The column was eluted with the same solution at a flow rate of 18 mL/hr and the aliquots of 3 mL were collected. The contents of hexose and hexuronic acid were determined based on Dubois *et al.* (19) and carbazole reactions (20).

Statistical analysis Data were expressed as means± standard deviation (SD) from 3 independent experiments. The statistical significance of differences between groups was determined by applying Student *t*-test. Significance of differences is indicated as a p values. Values of $p<0.05$ were considered statistically significant.

Results and Discussion

Evaluation of 3T3-L1 adipocyte differentiation The differentiation of preadipocyte into adipocytes is regulated by transcription factors such as PPARγ, C/EBPα, and leptin. The mRNA expression of those transcription factors can be detected by RT-PCR using specific primers as described in materials and methods. Because the housekeeping gene, G3PDH, is expressed with similar amounts of RNA in cells, the inhibitory effects of samples on differentiation of adipocyte can be measured through the expression level of the PPARγ and C/EBPα genes, 2 important adipogenic transcription factors. Therefore, in this study, we investigated the effects of polysaccharides from *hinmogi* on the expression of adipogenic transcription factors and adipogenic genes.

The polysaccharides from *hinmogi* Inhibit the expression of transcription factors, PPARγ, in 3T3-L1 adipocyte cells The 3T3-L1 cells were treated with polysaccharide P20, P40, P60, and P80 from *hinmogi* during differentiation and the cell lysates were subjected to Western blot using anti-PPARγ antibody as described in materials and methods. As shown in Fig. 1, the control 3T3-L1 cells without treatment of polysaccharides showed the strong immuno-reactive band in 67 kDa position, PPARγ1 (lane a), whereas the cells treated with polysaccharides were significantly decreased in expressions of PPARγ (lane b, c, d, e, and f). It should be noted that P80 showed the highest inhibitory effect among the polysaccharides fractionated from WSF on differentiation of 3T3 L1 adipocyte cells (lane f). Therefore, we concentrated our further studies in P80.

The P80 from *hinmogi* reduced the lipid contents in 3T3-L1 adipocyte cells It was reported that the number of larger lipid droplets is increased during differentiation from preadipocytes to adipocytes in 3T3-L1 cells (2). To measure the lipid contents in cells, triglyceride accumulation assay using INT kit (Fig. 2) and Oil Red-O staining (Fig. 3) were performed in 3T3-L1 cells treated with polysaccharides from mushrooms. As shown Fig. 2, treatment of P80, WSF and *maitake* X-fraction reduced triglyceride accumulation in 3T3-L1 cells during differentiation, in which the reductions were clearly shown at 2 mg/mL of 3 polysaccharides but were not significant in 1.0 and

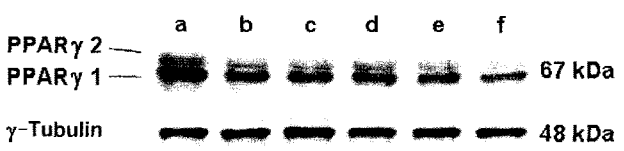

Fig. 1. Effects of polysaccharides from edible mushroom, *Tremella fuciformis*, on the protein abundance of lipogenic gene products of peroxisome proliferators-activated receptory (PPARγ) in 3T3-L1 cells. Non-treated cells (a); cells treated with WSP (b), P20 (c), P40 (d), P60 (e), and P80 (f).

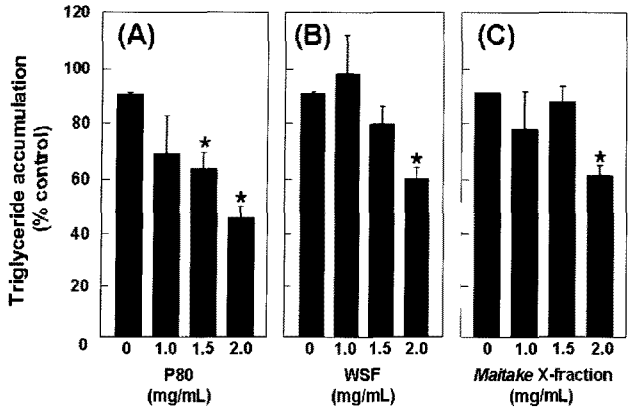

Fig. 2. Triglyceride accumulation in 3T3-L1 cells. The values are shown as means±SD from 3 independent experiments. The significance of each difference is indicated as a p value. *$p<0.05$ vs. control.

1.5 mg/mL of polysaccharides. Treatment with P80 on 3T3-L1 cells demonstrated the significant inhibitory effect on triglyceride accumulation, in which the degree of triglyceride accumulation in the cell was 69 and 49% of that of non-treated cells at 1.5 and 2 mg/mL, respectively (Fig. 2A). Whereas triglyceride contents of 3T3-L1 cells treated with WSF was 88 and 65% (Fig. 2B), and that with *maitake* X-fraction was 97 and 66% of that of non-treated cells at 1.5 and 2 mg/mL (Fig. 2C), respectively.

The inhibitory effect of P80 on triglyceride accumulation was confirmed by Oil Red-O staining, in which the cells treated with P80 contained less lipids than control cells (Fig. 3A). The degree of Oil Red-O staining in 3T3-L1 cells treated P80 was 34 and 22% of that of control cells at 1.5 and 2 mg/mL, respectively (Fig. 3B). Compare to that of non-treated cell, however, the degree of Oil Red-O staining of cells treated with WSF (Fig. 3C) and *maitake* X-fraction (Fig. 3D) were decreased to 50 and 51% at 1.5 mg/mL, and to 34 and 38% at 2.0 mg/mL, respectively. These results suggested that P80 had strong inhibitory effect on differentiation of 3T3-L1 preadipocytes into adipocytes.

The P80 from *hinmogi* inhibit the differentiation of 3T3-L1 adipocyte cells through inhibition of mRNA expression of PPARγ, C/EBPα, and leptin To investigate the mechanism of inhibitory effects of P80 on adipocyte differentiation, mRNA expression levels of 2 important differentiation makers, PPARγ and C/EBPα, were used to monitor the progress of differentiation in the preadipocytes in the presence or absence of P80. The results demonstrated that mRNA expressions of PPARγ (266 bp) in 3T3-L1 cells treated with 2 mg/mL of P80 were significantly reduced compared to that in non-treated cells, and in cells treated with WSF or *maitake* X-fraction (Fig. 4A). Furthermore, P80 showed a significant inhibitory effect on mRNA expression of PPARγ at 2 mg/mL, which was clearly higher than WSF, and *maitake* X-fraction, a well known anti-diabetic substance.

The mRNA expressions profiles of C/EBPα (238 bp) in 3T3-L1 cells treated with P80, WSF and *maitake* X-fraction were shown in Fig. 4B. The P80 showed a significant reduction in mRNA expression of C/EBPα at 2 mg/mL, whereas all cells showed similar amounts of mRNA expression of house keeping gene, G3PDH (738 bp). The significantly reduced mRNA levels of major

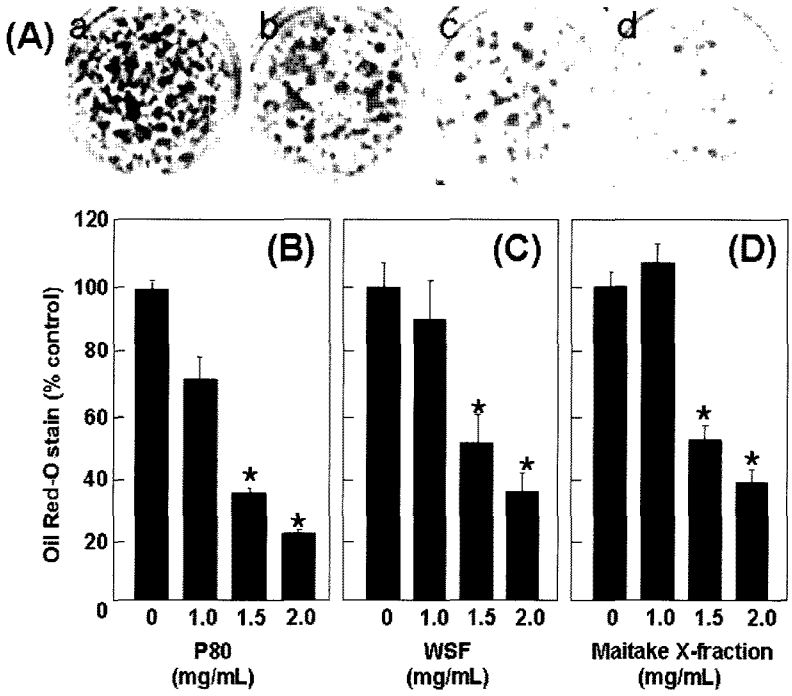

Fig. 3. Effect of mushroom polysaccharides on accumulation of neutral lipids (A) and triglycerides (B, C, and D) during differentiation of 3T3-L1 adipocytes in culture. The values are shown as means±SD from 3 independent experiments. The significance of each difference is indicated as a p value. *$p<0.05$ vs. control.

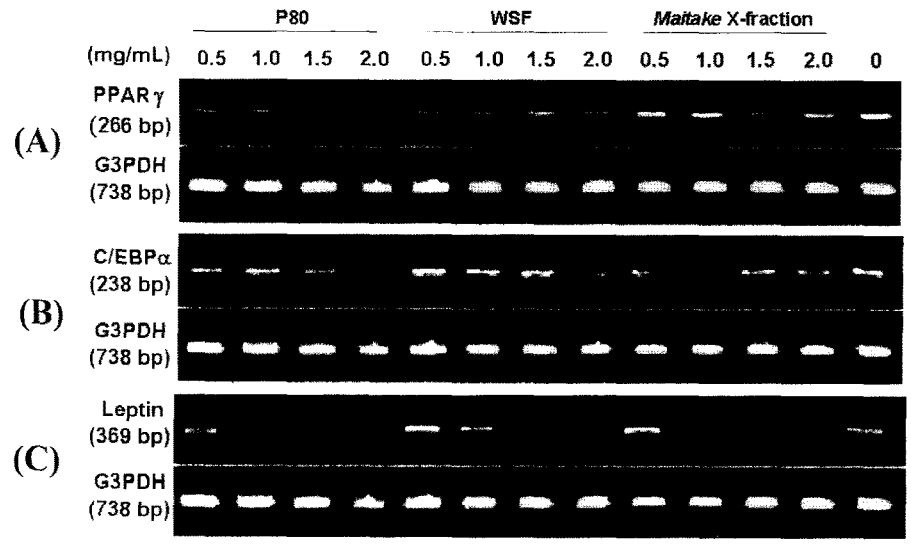

Fig. 4. Effects of polysaccharides on the mRNA expression of PPARγ (A), C/EBPα (B), and leptin (C) in 3T3-L1 cells. G3PDH: Glyceroaldehyde-3-phosphate dehydrogenase.

adipogenic transcription factors, PPARγ and C/EBPβ in 3T3-L1 cells treated with P80 suggested that P80 inhibited the adipogenesis by reducing transcription of PPARγ and C/EBPα. Myostatin, a new TGF-beta family member and known as a muscle growth inhibitor, inhibited preadipocyte differentiation in 3T3-L1 cells, which was mediated, in part, by altered regulation of C/EBPα and PPARγ (21). It was reported that the expression of C/EBPβ in 3T3-L1 cells was induced early in the presence of Tontonoz methylisobutyxanthine (MIX) and then C/EBPβ induced PPARγ and C/EBPα, followed by the expression of adipogenic genes such as G3PDH and aP2 to accumulate fat into cells (22). Leptin, a hormone produced primarily by adipocytes, regulates energy expenditure and food intake, and its expression is regulated by physiological states such as fasting and feeding, and as well as hormonal regulation. In this experiment, the inhibitory effects of polysaccharides from *hinmogi* on the mRNA expression of leptin in 3T3-L1 cells was determined using RT-PCR because leptin expression in 3T3-L1 cells is generally too low to detect using Northern blot analysis (23). The results demonstrated that mRNA expressions of leptin (369 bp) in 3T3-L1 cells treated with P80, WSF and *maitake* X-fraction were significantly inhibited (Fig. 4C). Most notably, the cells treated with P80 were completely inhibited in leptin expression at 2 mg/mL.

The morphology of 3T3-L1 cells treated with or without P80 was observed (Fig. 5). The 3T3-L1 cells exhibited spindle shape in the stage of the adipocyte (Fig. 5a). The cells which had undergone differentiation without P80 treatment showed significantly increased lipid droplets (Fig. 5b). The cells induced to differentiate with the

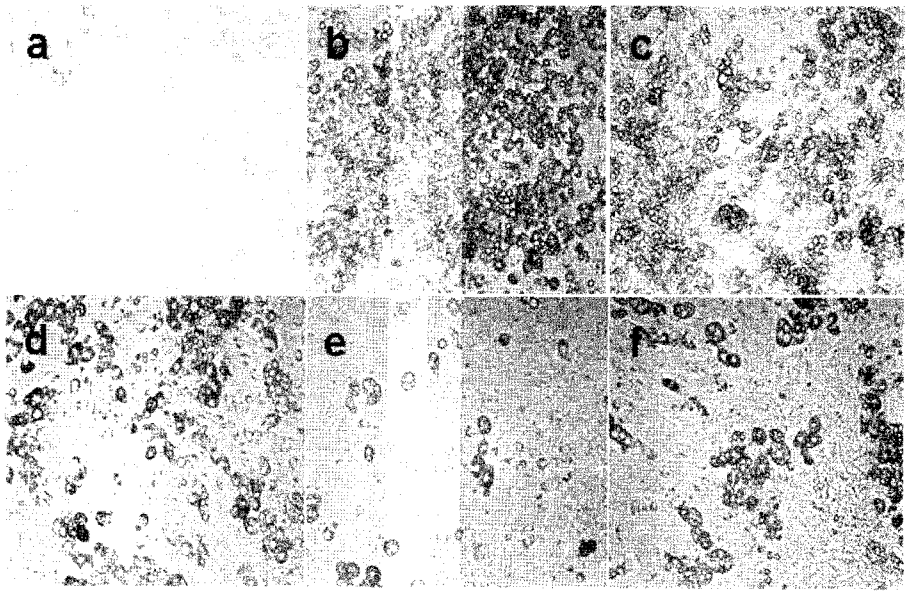

Fig. 5. Photomicrographys of 3T3-L1 cells. Undifferentiated cells (a); differentiated cells with treatment of 0 (b), 0.5 (c), 1.0 (d), 1.5 (e), and 2.0 mg/mL (f) of P80.

treatment of P80, however, showed a reduction in the formation of lipid droplets (Fig. 5c, d, e, and f). These results are consistent with the triglyceride accumulation assay and Oil Red-O staining (Fig. 2 and 3).

When P80 was applied on Sephacry S-400 column (2× 170 cm) equilibrated with 0.5 M ammonium bicarbonate (pH 7.0), a single symmetric peak was observed, containing both neutral sugar and hexuronic acid. The P80 has an average molecular mass of about 480 kDa on Sephacry S-400 column, previously calibrated using dextran with different molecular masses (data not shown).

We demonstrated that P80 inhibited the adipocyte differentiation in 3T3-L1 cells through down regulation of adipocyte-specific transcription factor such as PPARγ and C/EBPα, and of hormone produced primarily by adipocytes such as leptin, which was confirmed by a decrease in lipid accumulation. It has been reported that TGFβ1 (transforming growth factor-β1)-induced SAPK/JNK (stress-activated protein kinases/c-Jun N-terminal kinase) phosphorylation leads to downstream signaling which negatively affect the activity of PPARγ (24), and CREB activation downregulated the expression of transcrip-tion factors such as C/EBPα, PPARγ, RXRα, and SREBP-1 in 3T3-L1 cells (25). A possible inhibitory mechanism of P80 on differentiation may come from downregulation of transcriptional signaling related to CREB and TGFβ1. It was reported that adipocyte differentiation and the extent of subsequent fat accumulation are closely related to obesity (2). Weight gain is also a frequent side effect of insulin therapy in type II diabetic patients (26). In this study, the inhibitory activity on differentiation was clearly shown only when high concentration of P80 was treated to 3T3-L1 adipocyte. However, further studies should be performed such as anti-differentiation mechanism, anti-obesity activity (27), and *in vivo* study (28) of P80 from *hinmogi*. Nevertheless, our findings may constitute a new source of compounds with potential to treat or prevent obesity.

Acknowledgments

We thank Jung-Hoon Kang, Department of Biochemistry, College of Medicine, The Catholic University of Korea, for help with the cell culture.

References

1. Harmon AW, Harp JB. Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis. Am. J. Physiol.-Cell. Ph. 280: C807-C813 (2001)
2. Kawada T, Takahashi N, Fushiki T. Biochemical and physiological characteristics of fat cell. J. Nutr. Sci. Vitaminol. 47: 1-12 (2001)
3. Wise LS, Green H. Participation of one isozyme of cytosolic glycerophosphate dehydrogenase in the adipose conversion of 3T3 cells. J. Biol. Chem. 254: 273-275 (1979)
4. Takahashi N, Kawada T, Yamamoto T, Goto T, Taimatsu A, Aoki N, Kawasaki H, Taira K, Yokoyama KK, Kamei Y, Fushiki T. Overexpression and ribozyme-mediated targeting of transcriptional coactivators CREB-binding protein and p300 revealed their indispensable roles in adipocyte differentiation through the regulation of peroxisome proliferators-activated receptor gamma. J. Biol. Chem. 277: 16906-16912 (2002)
5. Takahashi N, Kawada T, Goto T, Yamamoto T, Taimatsu A, Matsui N, Kimura K, Saito M, Hosokawa M, Miyashita K, Fushiki T. Dual action of isoprenols from herbal medicines on both PPARγ and PPARα in 3T3-L1 adipocytes and HepG2 hepatocytes. FEBS Lett. 514: 315-322 (2002)
6. Borchers AT, Keen CL, Gershwin ME. Mushrooms, tumors, and immunity: An update. Exp. Biol. Med. 229: 393-406 (2004)
7. Yoon S-J, Yu M-A, Pyun Y-R, Hwang J-K, Chu DC, Juneja LR, Mourao PAS. The nontoxic mushroom *Aurucularia auricula* contains a polysaccharide with anticoagulant activity mediated by antithrombin. Thromb. Res. 112: 151-158 (2003)
8. Bellini MF, Angeli JP, Matuo R, Terezan AP, Ribeiro LR, Mantovani MS. Antigenotoxicity of *Agaricus blazei* mushroom organic and aqueous extracts in chromosomal aberration and cytokinesis block micronucleus assays in CHO-k1 and HTC cells. Toxicol. In Vitro 20: 355-360 (2006)
9. Guo FC, Kwakkel RP, Williams BA, Parmentier HK, Li WK, Yang ZQ, Verstegen MW. Effects of mushroom and herb polysaccharides on cellular and humoral immune responses of *Eimeria tenella*-infected chickens. Poultry Sci. 83: 1124-1132 (2004)
10. Guo FC, Kwakkel RP, Williams CB, Suo X, Li WK, Verstegen MW. Coccidiosis immunization: Effects of mushroom and herb polysaccharides on immune responses of chickens infected with *Eimeria tenella*. Avian Dis. 49: 70-73 (2005)
11. Ukai S, Kiriki H, Nagai K, Kiho T. Synthesis and antitumor activities of conjugates of mitomycin C-polysaccharide from *Tremella fuciformis*. Yakuga. Zasshi 112: 663-668 (1992)
12. Kiho T, Tsujimura Y, Sakushima M, Usui S, Ukai S. Polysaccharides in fungi. XXXIII. Hypoglycemic activity of an acidic polysaccharide (AC) from *Tremella fuciformis*. Yakuga. Zasshi 114: 308-315 (1994)
13. Kim K-A, Chang H-Y, Choi S-W, Yoon J-W, Lee C. Cytotoxic effects of extracts from *Tremella fuciformis* strain FB001 on the human colon adenocarcinoma cell line DLD-1. Food Sci. Biotechnol. 15: 889-895 (2006)
14. Yoon S-J, Pereira MS, Pavao MSG, Hwang J-K, Pyun Y-R, Mourao PAS. The medicinal plant *Porana volubilis* contains polysaccharides with anticoagulant activity mediated by heparin cofactor II. Thromb. Res. 106: 51-58 (2002)
15. Rubin CS, Hirsh A, Fung C, Rosen OM. Development of hormone receptors and hormonal responsiveness *in vitro*: Insulin receptors and insulin sensitivity in the preadipocyte and adipocyte forms of 3T3-L1 cells. J. Biol. Chem. 253: 7570-7578 (1978)
16. Wu Z, Bucher NL, Farmer SR. Induction of peroxisome proliferator-activated receptor γ during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPβ, C/EBPζ, and glucocorticoids. Mol. Cell Biol. 16: 4128-4136 (1996)
17. Vankoningsloo S, Piens M, Lecocq C, Gilson A, De Pauw A, Renard P, Demazy C, Houbion A, Raes M, Arnould T. Mitochondrial dysfunction induces triglyceride accumulation in 3T3-L1 cells: Role of fatty acid beta-oxidation and glucose. J. Lipid Res. 46: 1133-1149 (2005)
18. Lin FT, Lane MD. Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes. Genes Dev. 6: 533-544 (1992)
19. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. A colorimetric method for determination of sugars. Nature 168: 167 (1951)
20. Dische Z. A new specific color reaction of hexuronic acid. J. Biol. Chem. 167: 189-198 (1947)
21. Kim H-S, Liang L, Dean RG, Hausman DB, Hartzell DL, Baile CA. Inhibition of preadipocyte differentiation by myostatin treatment in 3T3-L1 cultures. Biochem. Bioph. Res. Co. 281: 902-906 (2001)
22. Mandrup S, Lane MD. Regulating adipogenesis. J. Biol. Chem. 272: 5367-5370 (1997)
23. Kallen CB, Lazar MA. Antidiabetic thiazolidinediones inhibit leptin (*ob*) gene expression in 3T3-L1 adipocytes. P. Natl. Acad. Sci. USA 93: 5793-5796 (1996)
24. Hong KM, Belperio JA, Keane MP, Murdick MD, Strieter RM. Differentiation of human circulating fibrocytes as mediated by transforming growth factor-β and peroxisome proliferator-activated receptor γ. J. Biol. Chem. 282: 22910-22920 (2007)
25. Vankoningsloo S, Pauw AD, Houbion A, Tejerina S, Demazy C, de Longueville F, Bertholet V, Renard P, Remacle J, Holvoet P, Raes M, Arnould T. CREB activation induced by mitochondrial

dysfunction triggers triglyceride accumulation in 3T3-L1 preadipocytes. J. Cell Sci. 119: 1266-1282 (2006)
26. Laville M, Andreelli F. Mechanisms for weight gain during blood glucose normalization. Diabetes Metab. 26: 42-45 (2000)
27. Mun E-G, Soh J-R, Cha Y-S. L-Carnitine reduces obesity caused by high-fat diet in C57BL/6J mice. Food Sci. Biotechnol. 16: 228-233 (2006)
28. Chung M-J, Lee Y-S, Kim B-C, Lee S-B, Moon T-W, Lee S-J, Park K-H. The hypoglycemic effects of *Acarviosine*-glucose modulate hepatic and intestinal glucose transporter *in vivo*. Food Sci. Biotechnol. 16: 851-855 (2006)