Chinese Herbal Medicine Yi-Gan-San Decreases the Lipid Accumulation in Mouse 3T3-L1 Adipocytes by Modulating the Activities of Transcription Factors SREBP-1c and FoxO1

Masayuki Izumi,^{1,2} Takashi Seki,² Koh Iwasaki² and Kazuichi Sakamoto¹

¹Graduate school of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan

²Center for Asian Traditional Medicine, Graduate School of Medicine, Tohoku University, Miyagi, Japan

Abnormal lipid metabolism in adipose tissue is closely related to the occurrence and progression of a wide variety of metabolic syndromes. We have analyzed the pharmacological effects of Chinese herbal medicines on cell differentiation and lipid metabolism in adipocytes. Yi-Gan-San (YGS) is a Chinese herbal medicine that is effective in treating the behavioral and psychological symptoms of dementia; however, its physiological mechanism remains unclear. We analyzed the effects of YGS on lipid accumulation in mouse 3T3-L1 adipocytes. Adipocyte differentiation was induced in mouse 3T3-L1 preadipocytes by treatment with the mixture of dexamethasone, 3-iso-butyl-1-methylxanthine, and insulin, and cells were cultured for 8 days with Chinese herbal medicines, including YGS. YGS effectively reduced the lipid accumulation in the differentiated 3T3-L1 cells in a dose-dependent manner, but had no effect on cell viability. YGS also reduced the activity of glycerol-3-phosphate dehydrogenase, an enzyme involved in lipid synthesis. In contrast, YGS gave no noticeable effect on glucose uptake and fatty acid uptake in the differentiated 3T3-L1 cells. Moreover, we established the stably transfected 3T3-L1 cell lines, each of which expresses the luciferase reporter gene under the control of sterol regulatory element-binding protein-1c (SREBP-1c) or FoxO1. SREBP-1c is a transcription factor involved in fatty acid synthesis, and FoxO1 is a forkhead-type transcription factor involved in adipocyte differentiation. Using these cell lines, we showed that YGS reduced the transcriptional activity of SREBP-1c, whereas YGS increased the activity of FoxO1. Thus, YGS may suppress lipid synthesis and fat accumulation in adipocytes through modulating the activities of SREBP-1c and FoxO1. ——— Yi-Gan-San; SREBP-1c; FoxO; Adipocyte; 3T3-L1.

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The abnormal lipid metabolism in adipose tissue is strongly associated with the occurrence and progression of metabolic syndromes (MS), including Diabetes mellitus (DM) (Shah et al. 2008; Kamada et al. 2008; Eckardt et al. 2008). Recently, it was reported that a disorder of lipid metabolism is also involved in aggravating of Alzheimer's disease (AD) (Ott et al. 1999; Kivipelto et al. 2005; Luchsinger and Mayeux 2007). On the other hand, the adipocyte secretes bioactive substances and growth factors, under the control of other organs, and plays an important role as an internal secretion cell for the homeostasis maintenance of the living body (Wozniak et al. 2009; Rasouli and Kern 2008). Therefore, analysis of lipid metabolism and adipocytokine-related gene expression in adipocytes is critical to the basic research for the prevention and treatment of these diseases.

A transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c), is enriched in the liver and adipocyte, and it is involved in the synthesis of fatty acids and

cholesterol and is a main regulator of lipid metabolism under the control of the insulin signal cascade (Yang et al. 2007). Three isoforms, SREBP-1a, SREBP-1c, and SREBP-2, form the SREBP family (Eberlé et al. 2004). SREBP-1c has also been associated with the occurrence of DM, obesity and AD (Kotzka and Müller-Wieland 2004; Kelicen and Nordberg 2006; Bonzón-Kulichenko et al. 2009). In patients with lipid metabolic disorder, Reactive Oxygen Species (ROS) level in the blood is a little higher than normal people (Mecocci et al. 2002; Potenza et al. 2009). We have reported that ROS promotes lipid accumulation through the activation of SREBP-1c (Sekiya et al. 2008). Therefore, we focused on SREBP-1c as a key factor for understanding the mechanism of lipid metabolic disease. We also focused on FoxO (Forkhead box class O), the forkhead-type transcription factor that promotes the expression of superoxide dismutase (SOD), catalase and other cell-survival related genes, which are part of the insulin-signaling cascade (Kops et al. 2002; Curtis et al. 2007; Erol 2009).

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Correspondence: Kazuichi Sakamoto, Graduate school of Life and Environmental Sciences, University of Tsukuba, Ibaraki, 305-8572 Japan.

e-mail: sakamoto@biol.tsukuba.ac.jp

Chinese herbal medicines are effective against various diseases, such as dementia, pneumonia, and arteriosclerosis, and have few side effects (Iwasaki et al. 2004, 2007; Zhang et al. 2008). In particular, *Yi-Gan-San* (YGS, Japanese name: Yokukansan) has been used to treat neurosis and insomnia (Miyaoka et al. 2008). Recently, it was reported that YGS is effective against the behavioral and psychological symptoms of dementia (BPSD) (Iwasaki et al. 2005; Mizukami et al. 2008). Although psychoactive drugs and antipsychotics are commonly used for the treatment of BPSD, antipsychotics possibly increase the risk of death among the elderly (Wang et al. 2005), and increase body weight and insulin resistance (Nasrallah 2003; Minet-Ringuet et al. 2006) as side effects. Compared to these medicines, Chinese herbal medicines generally have lesser side effects. Among them, YGS is particularly effective for cell protection and maintenance of cellular function (Tateno et al. 2008; Egashira et al. 2008). So far, there is no report that YGS is involved in the aggravation of lipid metabolism. Though the clinical effect of Chinese herbal medicine is obvious, the pharmacological effect to the cultured cell and its molecular mechanism are little known.

To understand the role of YGS in lipid metabolism, we analyzed the pharmacological effects of lipid accumulation and biochemical effects to transcription factor SREBP-1c by using mouse 3T3-L1 preadipocytes. Rikkunshito (RST) (Takeda et al. 2008) and Hochuekkito (HET) (Matsumoto et al. 2007), which have different effect to YGS, were used as comparative medicines.

Materials and Methods

Cell culture

3T3-L1 cell line was obtained from the Health Science Research Resources Bank (HSRRB, Osaka, Japan). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM High-glucose) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% FBS (Sanko Junyaku, Tokyo, Japan). Cells were cultured for 2 days to confluence then for a further 2 days in media supplemented with 0.25 mM dexamethasone (Sigma-Aldrich), 0.5 mM 3-iso-butyl-1-methylxanthine (Sigma-Aldrich), and 10 μ g/ml insulin (Wako, Osaka, Japan) (DMI induction). Then, cells were cultured for 2 days in media containing 5 μ g/ml insulin, and for 4 days in standard culture media.

Chinese herbal medicine

Yi-Gan-San (YGS) (TJ-54. Tsumura, Tokyo, Japan), Rikkunshito (TJ-43. Tsumura) and Hochuekkito (TJ-41. Tsumura) were kindly provided from Tsumura Co. Ltd. Five grams of these powders were dissolved in 50 ml double distilled water, autoclaved and centrifuged at 12,000 $\times$ G for 20 min (RS-2IV. TOMY SEIKO, Tokyo, Japan). The supernatant was filter-sterilized (20 μ m. Sartorius, Göttingen, Germany).

Oil-Red-O staining

To know the effect of YGS on lipid accumulation, we performed Oil-Red-O stain analysis. After washing cells with PBS, cells were fixed with 4% paraformaldehyde (Kantou Chemistry, Tokyo, Japan) for 1 h and then stained with 3 mg/ml Oil-red-O (in 60% isopropanol)

for 10 min. Cells were washed with sterile water then observed under a microscope (DMIRBE M2FLIII, Leica Microsystems Inc., Bannockburn, IL, USA). In addition, cells were stained with 0.3 mg/ml Oil-red-O, and the dye was eluted for 10 min with 100% isopropanol. The absorbance (O.D. 420 nm) was measured by a DU640 Spectrophotometer (Beckman, California, USA).

MTT assay

To know the effect of YGS on cell viability, we performed the MTT analysis. 3T3-L1 cells were plated on a 96-well plate at 1×10^4 cells/well and cultured for 4 days. Immediately after DMI induction, cells were cultured with 1,000 μ g/ml of YGS for an additional 8 days. After the culture, cells were washed with PBS, and incubated with 250 μ g/ml MTT solution (5 mg Thiazolyl Blue Teyrazolim Bromide, Sigma-Aldrich) for an additional 4 h. The product was dissolved in 0.04 N HCl in isopropanol, and the absorbance at 570 nm was measured.

Trypan Blue staining

To know the effect of YGS on cell viability, we performed the Trypan Blue analysis. 3T3-L1 cells were plated on 60-mm plates at 3×10^5 cells/well and cultured for 4 days. Immediately after DMI induction, cells were cultured with 1,000 μ g/ml of YGS for an additional 8 days. After the culture, cells were washed with PBS, and then incubated with 1 ml of 0.5 $\times$ trypan blue solution (Trypan Blue, Sigma-Aldrich) for 1 min. Cells were washed with PBS and observed under an optical microscope. Cell viability was calculated by the number of living cell and dead cell.

Glucose transport

To know the effect of YGS on glucose transport, we performed Glucose uptake analysis. The cells were cultured with YGS for 8 days, and then treated with 10 ml KRP-H buffer (131.2 mM NaCl, 4.7 mM KCl, 2.5 mM NaH_2PO_4 , 2.5 mM CaCl_2 , 1 mM MgSO_4 , 1 mM HEPES (pH 7.4) containing 10 mM 2-NBDG (2-(N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl)amino)-2-deoxyglucose) (Invitrogen Life Technologies, Carlsbad, CA, USA) at 37°C for 10 min. After washing the cells with KRP-H buffer, cells were observed under a LEITZ DMRXA HC RXA-6 fluorescence microscope (Leica, Solms, Germany).

Fatty acid transport

To know the effect of YGS on fatty acid transport, we performed Fatty acid uptake analysis. The cells were cultured with YGS for 8 days, and then treated with PBS ($\text{Ca}^{2+}/\text{Mg}^{2+}$) containing 20 mM BODIPY3823 (Invitrogen) and 20 mM BSA (fatty acid free, Wako) at 37°C for 2 min. After washing the cells twice with PBS containing 20 mM BSA, cells were observed under a LEITZ DMRXA HC RXA-6 fluorescence microscope (Leica).

Glycerol-3-phosphate dehydrogenase activity

To know the effect of YGS on lipid synthesis, activity of glycerol-3-phosphate dehydrogenase (GPDH), an enzyme involved in lipid synthesis, was analyzed. 3T3-L1 cells were plated on 60-mm plates at 3×10^5 cells/well and cultured for 4 days. Immediately after DMI induction, cells were cultured with 1,000 μ g/ml of YGS for an additional 8 days. Then cells were treated with GPDH buffer (0.28 M Sucrose 9.58g, 5 mM Tris-HCL 500 μ l, 1 mM EDTA 200 μ l, dDW 99.1 ml, 0.002% β -ME 200 μ l) and 10 $\times$ protein inhibitor (1 mM

AEBSF (SIGMA), 130 μ M Bestatin (Wako), 14 μ M E-64 (SIGMA)), 1 mM EDTA (Wako, 1 μ M pepstatin A (SIGMA). After homogenization (0.5-Amplitude; 60), cell lysates were centrifuged (15,100 $\times$ G, 4°C, 10 min). The supernatant was mixed with GPDH reaction buffer

(TAE solution 5 μ l, 5 mM dehydroxy aceto phosphatic acid 10 μ l, 0.5 mM NADH, dDW 51 μ l), and O.D. (340 nm) was measured.

Table 1. Primers for RT-PCR. PCR was performed using the primers indicated below under optimal amplification conditions (95°C for 10 min; 35-38 cycles of 95°C for 30 sec, 51-58°C for 30 sec, 72°C for 30 sec; 72°C for 7 min) for each gene. The PCR amplification of each cDNA was performed independently using three samples in triplicate.

Gene	Sense	Anti-sense
SREBP-1c	ACTTCTAGACGGGCGCCCATGGAGACT	ACTTCTAGATCCTCAAAGGCTGGA
PGC-1a	GCAGAAGAGCCGTCTCTA	CCTGCAGTTCAGAGAGAT
UCP2	CCTGATTGCAGATCTCAA	AGGTCATAGGTCACCAGC
PPAR γ	AATAGATCTTGATTAGGAGTTTCA	ATTAAGCTTACCATGCTCTGGGTC
Adiponectin	GGACATCCTGGCCACAAA	GTCCTTCTTGAAGAGGCT
Adipsin	CTGCTGGACGAGCAGTGG	GATGACACTCGGGTAT
CD36	GCAGAAGAGCCGTCTCTA	AGGTCGATTGAGATCCGC
GLUT4a	TGCTGGGCACAGCTAGCTACC	TATGGCCACGATGGAGAC
C/EBP	CAGCGGCAGCCTGTCCAC	CACGTGTGTTGCGTAGTC
Foxo1	GATCTACGAGTGGATGGT	CAGCGTAGACGCCATCTT
SIRT1	TAATCAGGTAGTTCTCG	AAAGTTTGGCATATTCAC
MnSOD	CCCATCTCCACTAAAAAT	CAGCCTCCCAGTAGCTG
Cu/ZnSOD	TTAACTGAAACCAGCATC	CAGATGATGCTGAGACTC
Catalase	TTGGCCTCACAAGGACTA	GGCAATGTTCTCACACAG
β -actin	CCGGGACCTGACGGACTACCTCATGAAGAT	AATAGTGATGACTTGGCCGTCAGGCAGCTC

Fig. 1. Effect of Chinese herbal medicines on lipid accumulation. (A) 3T3-L1 cells were cultured in 48-well plates at 2×10^4 cells/well for 4 days. Differentiation was induced with DMI, and the cells were then immediately cultured with 1000 μ g/ml of YGS, RST, or HET for an additional 8 days. Untreated cells [control (-)] and double distilled water (1%)-treated cells [control (dDW)] were used as negative controls. Cells were stained with Oil-red-O to label lipids. (B) After Oil-red-O staining, the dye was eluted from the cells with isopropanol and quantified, and the results were plotted. * $P < 0.05$, (C) 3T3-L1 cells were cultured in 96-well plates at 1×10^4 cells/well and treated with YGS, RST, or HET for 8 days. After the culture, cell viability was assessed with an MTT assay ($n = 3$ cultures/condition).

Luciferase reporter assay

To know the effect of YGS on the activity of each transcription factors, we performed Luciferase reporter analysis. We prepared the DNA fragments that carry the consensus sequence (Insuline Responsive Sequence 3; IRS3) or the genomic sequence (catalase promoter region: Catalase) of FoxO binding site, and the consensus sequence (SREBP-1c Responsive Element: SRE) of SREBP-1c binding site. The luciferase reporter plasmids with a FoxO DNA-binding site (IRS3-Luc, Catalase-Luc) and with a SREBP-1c DNA-binding site (SRE-Luc) were constructed. The stable 3T3-L1 cells that carry IRS3-Luc, Catalase-Luc or SRE-Luc were established (3T3-L1/IRS3-Luc, 3T3-L1/Catalase-Luc, 3T3-L1/SRE-Luc). The cells were cultured on 60-mm plates at 3×10^5 cells/well. After 8 days culture with YGS, cells were incubated with 450 μ l Reporter Lysis Buffer (Promega, Madison, WI, USA) for 60 min at -80°C . After brief centrifugation ($32,000 \times G$, 3 min) of the cell lysates, supernatant was collected and luciferase activity was measured with a Luminometer Micro Lumat LB96p (Berthold Technology, Germany), and plotted on a graph after normalization by cell number.

RT-PCR

To know the effect of YGS on gene expression, we performed

RT-PCR analysis. The cells were cultured with YGS for 8 days, and RNA was extracted by the standard GTC-acid phenol method (Chomczynski and Sacchi 1987). After cDNA synthesis with M-MLV reverse transcriptase (Takara, Shiga, Japan), PCR was performed with the primers indicated in Table 1. PCR (95°C for 10 min; 35–38 cycles of 95°C for 30 sec, $51\text{--}58^\circ\text{C}$ for 30 sec, 72°C for 30 sec; 72°C for 7 min) was performed using TP600 (Takara).

Statistical analysis

Results are expressed as mean $\pm$ standard deviation. Comparisons between groups were made by analysis of variance (ANOVA), were examined by Tukey's all-pairwise-comparison test. Differences were considered significant when $P < 0.05$. Statistical analysis was performed using Excel 2004 (Microsoft, Redmond, WA, USA) with the add-in software Statcal (Kamon et al. 2008).

Results

Effect of YGS on lipid accumulation

Adipocyte differentiation was induced in mouse 3T3-L1 preadipocytes by DMI treatment. YGS was then added to the cells, which were cultured for a further 8 days. Because our preliminary experiments revealed that YGS

Fig. 2. Effect of YGS on lipid accumulation. 3T3-L1 cells were cultured for 4 days, induced with DMI, and cultured with YGS (200, 500, or 1,000 $\mu\text{g/ml}$) for an additional 8 days. (A) Cells were cultured with YGS on 60-mm plates at 3×10^5 cells/plate, then counted ($n = 6$ cultures/condition). (B) After Oil-red-O staining, the dye was extracted and quantified with a spectrophotometer. $*P < 0.05$ ($n = 9$ cultures/condition). (C) Cells were cultured in 96-well plates at 1×10^4 cells/well and cell viability was analyzed with an MTT assay ($n = 9$ cultures/condition). (D) Cells were cultured on 60-mm plates at 3×10^4 cells/plate. After culture with the indicated concentration of YGS, dead cells were detected by Trypan Blue staining, and cell viability was plotted ($n = 6$).

(100, 200, 300, 500, 1,000, 2,000 $\mu\text{g/ml}$) gave little effects to cell viability, we applied a maximum 1,000 $\mu\text{g/ml}$ of YGS, a dose which gave the most remarkable and reliable effects to lipid accumulation. We also applied 1,000 $\mu\text{g/ml}$ of Rikkunshito (RST) and Hochyuekkito (HET) to parallel cell cultures as controls. The cells were then stained with Oil-red-O to assess lipid accumulation. YGS significantly reduced lipid accumulation relative to that in controls (Figs. 1A, B), whereas RST and HET increased lipid accumulation. The results of an MTT assay showed that YGS and HET had no effect on cell viability (Fig. 1C), whereas RST slightly decreased cell survival. After 8 days of treatment with 200, 500, or 1,000 $\mu\text{g/ml}$ YGS, the total number of cells had not changed greatly (Fig. 2A), but YGS had reduced lipid accumulation in a dose-dependent manner (Fig. 2B). MTT and Trypan Blue staining assays confirmed that YGS had little effect on cell viability (Figs. 2C, D).

Effect of YGS on lipid accumulation in differentiated adipocytes

Eight days after DMI induction, we incubated 3T3-L1 cells with YGS for 2 days (days 8-10 after DMI treatment), 4 days (days 8-12), 6 days (days 8-14), or 8 days (days 8-16). YGS reduced lipid accumulation in differentiated adipocytes (Fig. 3A). Quantification of Oil-red-O in the cells confirmed that YGS treatment reduced lipid accumulation (Fig. 3B) without affecting cell viability (Fig. 3C).

Effect of YGS on GPDH activity and uptake of glucose and fatty acids

Immediately after DMI induction, we added 1000 $\mu\text{g/ml}$ YGS to the cell culture. To assess the incorporation of glucose and fatty acid after exposure to YGS, cells were treated with 2-NBDG or BODIPY3823. YGS had no effect on glucose uptake (Fig. 4A) or fatty acid uptake (Fig. 4B). Immediately after DMI induction, we added 1,000 $\mu\text{g/ml}$ YGS to the cell culture and analyzed the enzymatic activity of glycerol-3-phosphate dehydrogenase (GPDH). YGS significantly reduced GPDH activity (Fig. 4C).

Effect of YGS on transcriptional activity of SREBP-1c and FoxO

3T3-L1 cells were stably transfected by luciferase reporter plasmids carrying the FoxO (IRS3-Luc, Catalase-Luc) and SREBP-1c (SRE-Luc) DNA binding sites respectively. We treated these cells with 200, 500, or 1000 $\mu\text{g/ml}$ YGS for 8 days and analyzed the luciferase activity. The luciferase reporter assay revealed the direct activity of transcription factor, which acts on the specific luciferase reporter plasmid, leading to the change of expression and resultant enzyme activity of luciferase. Transcriptional activity of SREBP-1c was decreased with increasing concentration of YGS (Fig. 5A), whereas transcriptional activity of FoxO was increased (Figs. 5B, C).

Fig. 3. Effect of YGS on lipid accumulation in differentiated adipocytes. 3T3-L1 cells were cultured for 4 days, treated with DMI, cultured for further 8 days, and then treated with YGS (1,000 $\mu\text{g/ml}$) for 2 days (days 8-10 after DMI treatment), 4 days (days 8-12), 6 days (days 8-14), or 8 days (days 8-16). After Oil-red-O staining, (A) cells were observed with a microscope, and (B) the dye was eluted and quantified (O.D. 420 nm) ($n = 9$ cultures/condition). (C) Cell viability was analyzed by MTT assay ($n = 9$ cultures/condition).

Fig. 4. Effect of YGS on GPDH activity and uptake of glucose and fatty acids. 3T3-L1 cells were cultured on 60-mm plates at 3×10^5 cells/plate for 4 days, induced to differentiate with DMI, and cultured with 1,000 µg/ml of YGS for an additional 8 days. To know the effect of YGS on glucose transport and fatty acid transport, cells were treated with 10 mM 2-NBDG and 20 mM BODIPY3823, respectively. After the culture with YGS, (A) glucose uptake and (B) fatty acid uptake were analyzed. (C) GPDH activity was analyzed in YGS-treated cells. (** $P < 0.01$, $n = 3$)

Effect of YGS on gene expression

We next used RT-PCR to analyze gene expression in 3T3-L1 cells treated with 200, 500, or 1,000 µg/ml YGS. The mRNA level of SREBP-1c was substantially reduced (Fig. 6). Expression of the mRNAs of PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-α), UCP2 (uncoupling proteins-2) (Kakuma et al. 2000; Arumugam et al. 2008), PPAR-γ (peroxisome proliferator-activated receptor-γ) (Tjokropawiro 2006; Escibano et al. 2009), adiponectin (Migrenne et al. 2009), adipsin (Drevon 2005), FoxO1, SIRT1 (silent mating type information regulation 2 homolog) (Frescas et al. 2005), MnSOD, Cu/ZnSOD, and catalase (Tothova et al. 2007) increased, whereas expression of CD36 (Hickman et al. 2008), GLUT4 (glucose transporter 4) (Barros et al. 2008), and C/EBPs (CCAAT/enhancer-binding proteins) (Obregon 2008) did not change at all.

Discussion

We evaluated the pharmacological effects of a Chinese herbal medicine, YGS, on differentiation and adipogenesis of mouse 3T3-L1 preadipocytes. YGS reduced lipid accumulation in differentiated 3T3-L1 cells in a dose- and incubation period-dependent manner without affecting cell viability (Figs. 1, 2, 3). Because YGS also reduced the enzyme activity of GPDH (Fig. 4) and the gene expression and transcriptional activity of SREBP-1c (Figs. 5, 6), YGS likely reduced lipid accumulation by controlling lipid synthesis.

SREBP-1c is a key regulatory transcription factor that controls fatty acid synthesis under the regulation of the insulin signal cascade (Liu et al. 2008). SREBP-1c also increases insulin resistance in lipid metabolic diseases by suppressing insulin receptor substrate-2 (Shimano 2002). In addition to affecting the expression of SREBP-1c through

Fig. 5. Effect of YGS on transcriptional activity of SREBP-1c and FoxO. Stably transfected 3T3-L1 cells (3T3-L1/SRE-Luc, 3T3-L1/IRS3-Luc, 3T3-L1/Catalase-Luc) were cultured on 60-mm plates at 3×10^5 cells/plate for 4 days. Differentiation was induced with DMI, and the cells were immediately cultured with YGS (200, 500, 1,000 μg/ml) for an additional 8 days. After the culture with YGS, the cells were assessed for transcriptional activity of SREBP-1c (3T3-L1/SRE-Luc) (A), FoxO (3T3-L1/IRS3-Luc) (B) and FoxO (3T3-L1/Catalase-Luc) (C). * $P < 0.05$, ** $P < 0.01$ ($n = 3$ cultures/condition).

Fig. 6. Effect of YGS on gene expression. 3T3-L1 cells were cultured on 60-mm plates at 3×10^5 cells/plate for 4 days. Differentiation was induced with DMI, and the cells were immediately treated with YGS (200, 500, 1,000 μg/ml) for an additional 8 days. After the culture with YGS, RNA was extracted for RT-PCR of the indicated genes.

the insulin signal cascade, our results suggest that YGS affects the transcriptional activity of SREBP-1c itself.

Excess ROS promotes lipid accumulation (Hong and Lee 2009) and occurrence and progression of degeneration

of neuron disease through the accumulation of Aβ (Yatin et al. 1999; Nunomura et al. 2001; Ahtoniemi et al. 2008; Linseman 2008; Sensi et al. 2008), as it damages cells and tissues. YGS includes a variety of crude extracts

(Atractylodis Lanceae Rhizoma, Poria, Cnidii Rhizoma, Uncariae Uncis Cum Ramulus, Angelicae Radix, and Bupleuri Radix Glycyrrhizae Radix), of which Uncariae Uncis Cum Ramulus plays a role in cell survival through the activation of anti-oxidative enzymes (Mahakunakorn et al. 2005) and suppression of A β accumulation (Fujiwara et al. 2006). Our results indicated that YGS induces the expression of the genes encoding SOD and catalase (Fig. 5), which act as anti-oxidants. As it is known that FoxO family is involved in the regulation of expression of MnSOD and catalase (Olmos et al. 2009), our present data demonstrated the similar results (Fig. 4). Furthermore, we and other groups previously revealed that ROS promote lipid accumulation by stimulating SREBP-1c activity (Kohjima et al. 2007; Sekiya et al. 2008). These findings suggest that the anti-oxidative activity of YGS may have led to the suppression of SREBP-1c transcriptional activity. Recently, Wang et al. reported that resveratrol possibly attenuate fat deposition by inhibiting SREBP1 expression via the Sirt1-FOXO1 pathway (Wang et al. 2009).

YGS also promoted the gene expression (Fig. 6) and transcriptional activity (Fig. 5) of FoxO. Nakae et al. (2003) reported that overexpression of FoxO1 suppressed early adipocyte differentiation. However, we found that FoxO1 is essential for the promotion of adipocyte differentiation (Munekata and Sakamoto 2009), and, therefore, the physiological role of FoxO in adipocyte differentiation remains unclear. In the current study, YGS promoted the transcriptional activity of FoxO and increased the gene expression of FoxO, SOD, and catalase, suggesting that YGS affects lipid metabolism in adipocytes partly through the action of FoxO.

Most Chinese herbal medicines have stronger effects when applied for longer periods. Our results suggest that YGS controls the expression of multiple genes through the regulation of several transcription factors, leading to the maintenance of homeostasis. Further systematic analysis of Chinese herbal medicines and gene expression may facilitate mechanistic studies and the development of clinical applications.

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