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Piperine, a Component of Black Pepper, Inhibits Adipogenesis by Antagonizing PPARγ Activity in 3T3-L1 Cells

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ABSTRACT: This study investigated the antiadipogenic activity of black pepper extract and its constituent piperine in 3T3-L1 preadipocytes as well as the underlying molecular mechanisms. Both black pepper extract and piperine, without affecting cytotoxicity, strongly inhibited the adipocyte differentiation of 3T3-L1 cells. The mRNA expression of the master adipogenic transcription factors, PPARγ, SREBP-1c, and C/EBPβ, was markedly decreased. Intriguingly, mRNA levels of PPARγ target genes were also down-regulated. Moreover, a luciferase reporter assay indicated that pipierine significantly represses the rosiglitazone-induced PPARγ transcriptional activity. Finally, GST-pull down assays demonstrated that piperine disrupts the rosiglitazone-dependent interaction between PPARγ and coactivator CBP. Genome-wide analysis using microarray further supports the role of piperine in regulating genes associated with lipid metabolism. Overall, these results suggest that piperine, a major component of black pepper, attenuates fat cell differentiation by down-regulating PPARγ activity as well as suppressing PPARγ expression, thus leading to potential treatment for obesity-related diseases.

KEYWORDS: *Piper nigrum Linne, black pepper, piperine, adipogenesis, adipocytes, peroxisome proliferator-activated receptor-γ*

INTRODUCTION

Obesity is known to be the most prevailing and common health dilemma to be solved throughout the world. However, its complex etiology has been an obstacle for its treatment.[1] In general, obese condition is mainly caused by excess accumulation of lipid and triglycerides in adipose tissue, which plays a pivotal role in energy balance with regulation of lipid homeostasis, glucose uptake in blood, and endocrine function.[2] Under the condition of excess energy, adipose tissues increase the number of adipocytes from preadipocytes by inducing differentiation.[3] Therefore, it is very important to control adipogenesis for regulating energy homeostasis and obesity.

Adipogenesis is a well-organized process regulated by adipogenic transcription factors, such as peroxisome proliferator-activated receptor-γ (PPARγ), sterol regulatory element binding protein (SREBP) family, and CCAAT-enhancer binding protein (C/EBP) family.[4−6] Of these factors, PPARγ has been focused on its role in adipocyte differentiation. In addition to being induced during adipogenesis, it is both necessary and sufficient for the process.[7] Once bound to a ligand such as synthetic thiazolidinediones (TZDs), PPARγ forms a heterodimer with retinoid X receptor (RXR) and regulates the expression of specific subsets of genes containing a PPAR-response element (PPRE) in their promoters.[7] These genes include fatty acid binding protein 4 (Fabp4 or aP2), adipsin, and lipoprotein lipase (LPL).[7−10] Recently, many other PPARγ target genes have been supplemented by genome-wide microarray and ChIP analysis.[11−14]

Black pepper is a well-known spice used worldwide for hundreds of years. *Piper nigrum* Linne (PnL), the source of black pepper, has been traditionally used for cholera, dyspepsia, gastric ailments, and diarrhea as an oriental medicinal plant.[15−17] It also has been reported that PnL reduces triglyceride accumulation in liver and levels of glucose and lipid in the blood.[18,19] Piperine, a pungent ingredient of black pepper, is a major alkaloid-amine component of PnL extract.[20] In addition to many efficacies such as painkiller,[21] antioxidant,[22,23] antitumor,[24,25] and anti-inflammation activities,[26] piperine also plays an important role in reducing the levels of lipids and glucose in the blood,[27,28] and thus decreasing blood pressure.[29] However, the molecular mechanisms underlying the piperine effects remain largely unknown.

In this study, we investigated the role of PnL extract and its component piperine in adipogenesis and adipogenic gene expression using 3T3-L1 preadipocytes. In addition, we explored the molecular mechanism of the piperine effect by focusing on its role in PPARγ regulation. Overall, our results

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Table 1. Primer Sequences Used for qPCR

gene	forward primer	reverse primer
Pparg	TTTTCAAGGGTGCCAGTTTCAATCC	AATCCTTGGCCCTCTGAGAT
Srebp-1c	CTGAGGCAAAGCTGAATAAATCTGCTG	GTTCTCCTGCTTGAGTTTCTGGTTG
C/EBP	ACGAGCGCGCCATCGACTTC	GAAGCCCGGCTCCGCCTTG
adipsin	CCTGAACCCTACAAGCGATG	GGTTCCACTTCTTTGTCCTCG
Lpl	ATCCATGGATGGACGGTAACG	CTGGATCCCAATACTTCGACCA
aP2	AAAGACAGCTCCTCCTCGAAGGTT	TGACCAAATCCCCATTTACGC
Cd36	GTCCTGGCTGTGTTTGGAGG	CTTGGCTAGATAACGAACTCTGTA
Glut4	AGGGGCCTGCCCGAAAGAGT	CTGTTGGCTCAGCTGCAGCAC
Lxra	GCTGCCCAGCAACAGTGTAA	CTGCCGGGGTTGTACCTCCGT
Scd1	CATCCCATCGCCTGCTCTACC	GAAACCTGCCCTCCTGACTCTC

suggest that piperine could be a lead natural compound for the treatment of fat-related disorders.

MATERIALS AND METHODS

Reagents and Chemicals. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Oil Red O, dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), insulin, dimethyl sulfoxide (DMSO), piperine, rosiglitazone, GW9662, and To901317 were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS) and bovine serum (BS) were obtained from GIBCO (Grand Island, NY, USA).

Extraction of *P. nigrum* Linne. The crushed *P. nigrum* Linne (PnL), supplied by the Oriental Medical Hospital of Dongguk University (Kyungju, Korea), was extracted three times, each time with 3 volumes of methyl alcohol at 60 °C for 24 h. The extract was then cooled to room temperature, filtered, evaporated under a reduced pressure using a rotary evaporator, and dissolved in DMSO.

MTT Assays. 3T3-L1 cells were grown in DMEM supplemented with 10% (v/v) BS and 1% antibiotics/antimyotics (Invitrogen, Carlsbad, CA, USA) and plated at 500 cells/well in a 96-well tissue culture plate. Indicated concentrations of PnL extract and piperine were treated and incubated for 72 h. Fifty microliters of MTT reagent (2 mg/mL) was added and incubated in each well for 4 h. The MTT medium was then replaced with 0.2 mL of DMSO, and absorbance was read at 550 nm.

Adipocyte Differentiation of 3T3-L1 Cells. 3T3-L1 cells were grown in DMEM supplemented with 10% (v/v) BS and 1% antibiotics/antimyotics (Invitrogen) at 37 °C and 5% CO_2 to confluence. After 2 days, cells were induced to adipocyte differentiation (day 0) with 0.5 μM dexamethasone, 100 μM IBMX, and 1 μg/mL insulin with or without the indicated PnL extract or piperine in DMEM supplemented with 10% FBS. After 2 days, and every 2 days thereafter, cells were switched to fresh medium containing DMEM plus 10% FBS, 1 μg/mL insulin, and PnL extract or piperine. After 10 days, cells were harvested for further experiments.

Oil Red O Staining. 3T3-L1 cells differentiated for a total of 10 days were washed twice with PBS (pH 7.4) and then fixed with 2 mL of 10% formalin in PBS for 30 min at room temperature. The cells were washed twice with 2 mL of distilled water and stained with 0.5% Oil Red O (Sigma) for 10 min with gentle agitation. Excess stain was removed with 60% isopropanol, and cells were washed twice with distilled water before being photographed under a light microscope. Accumulated lipids were extracted in 2 mL of 100% isopropanol and measured by reading absorbance at OD 500 nm.

RNA Extraction and Real-Time Quantitative RT-PCR. Total RNA was extracted from differentiated 3T3-L1 cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The cDNA was synthesized with 1 μg total RNA using MMLV reverse transcriptase and random primers (Invitrogen). Quantitative real-time PCR (qPCR) reactions were performed using the iQ SYBR Green Supermix and Icycler CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA). The thermal cycle conditions were initial denaturation at 95 °C for 3 min, followed by 40 cycles at 95 °C for 15 s, 58 °C for 20 s, and 72 °C for 20 s. Cycle threshold (C_t) values were determined in 20−30 cycles. Primers used for qPCR are shown in Table 1. All expression levels were normalized using GAPDH as an internal standard in each well. Fold expression was defined as the fold increase relative to controls.

Luciferase Reporter Gene Assays. HEK293 cells were seed in a 12-well plate and cotransfected with PPARγ, PPRE-tk-luciferase reporter gene (PPARγ response element fused to thymidine kinase promoter localized upstream of luciferase gene), and SV-40-driven β-galactosidase expression vectors using LipofectAMINE (Invitrogen). After transfection overnight, cells were fed DMEM containing 5% charcoal-stripped FBS and incubated overnight in the presence of rosiglitazone (1 μM) and increasing concentrations of piperine. Luciferase activity was measured as described previously.[30]

Glutathione *S*-Transferase (GST) Pull-down Assays. Here, we used the notion that the N-terminal domain of CREB-binding protein (CBP) interacts with the ligand-binding domain of PPARγ only in the presence of its ligand.[31,32] The CBP−PPARγ interaction was monitored by a GST pull-down assay as previously described with minor modifications.[33] The His (6X)-tagged ligand-binding domain of PPARγ (amino acids 315−505) was purified using a HiTrap Chelating HP column (GE Healthcare, Piscataway, NJ, USA). GST and GST-fused CBP (amino acids 1−460 and 602−1095) were purified on glutathione−Sepharose beads (GE Healthcare). Glutathione−Sepharose beads were equilibrated with binding buffer [50 mM Tris-Cl, pH 7.5, 200 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 1 mM phenylmethanesulfonyl fluoride (PMSF), 0.1% Nonidet-40]. Approximately equimolar amounts of GST (0.5 μg) or GST−CBP (1 μg) were mixed with 0.2 μg of His-PPARγ in the binding buffer supplemented with 10 μM rosiglitazone and/or indicated concentrations of piperine, and the mixtures were incubated at 30 °C for 30 min. Pre-equilibrated glutathione−Sepharose beads were added, and further incubation was allowed for 1 h. The beads were washed three times with the binding buffer. Bound proteins were eluted with 40 μL of 2× SDS loading buffer by boiling for 10 min and visualized by Western blotting using anti-His antibody (Applied Biological Materials, Richmond, Canada).

Microarray and Data Analysis. Total RNA was extracted from 3T3-L1 cells treated with 50 μg/mL PnL extract or 50 μM piperine for 10 days under the adipogenesis condition. RNA quantity and quality were checked using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA samples with an RNA integrity number (RIN) of >9 were used for further analysis in a two-color microarray experiment using Agilent-Mouse-44k-4plex according to the manufacturer's instructions. Equal amounts of total RNA were amplified, labeled, hybridized, washed, and scanned. The LOWESS (locally weighted linear regression curve fit) and dye-swap normalization methods were applied to the ratio (Cy5/Cy3) of the signal intensities generated in the microarrays. Results were filtered, and the cutoff was set at increase and decrease of 1.5-fold change. Genes exhibiting differences in expression level were classified into Gene Ontology (GO)-based functional categories (http://www.geneontology.org), KEGG (http://www.genome.jp/kegg/), and DAVID Bioinformatics Resources (http://david.abcc. ncifcrf.gov/).

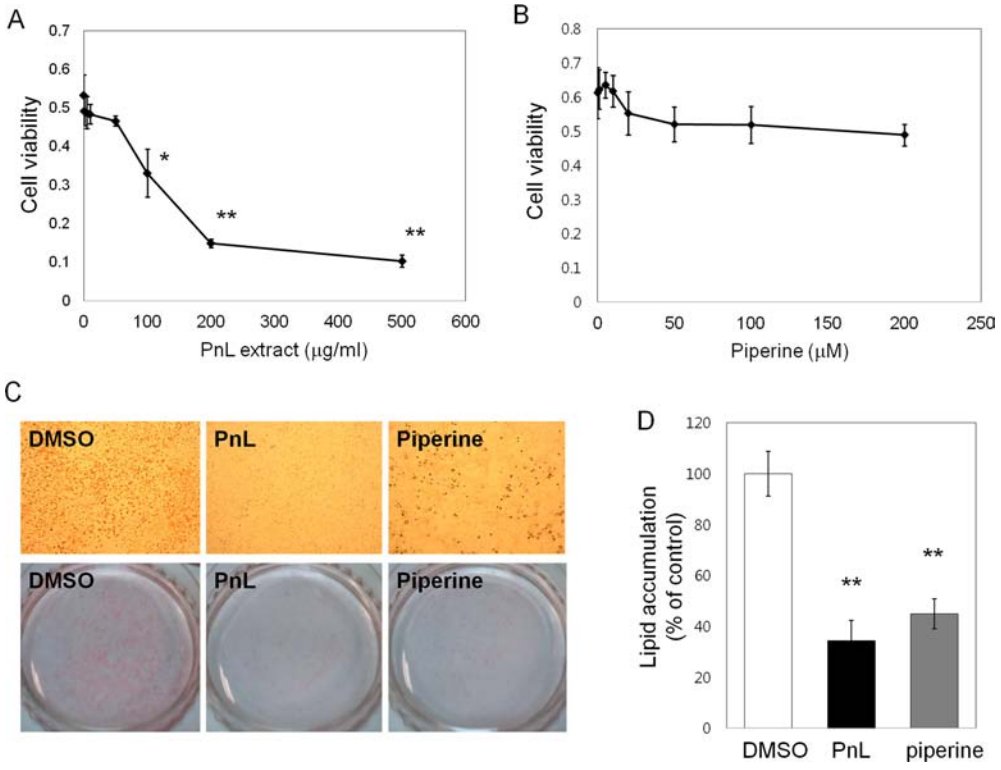

Figure 1. Effect of *Piper nigrum* Linne (PnL) extract and its component piperine on lipid accumulation in 3T3-L1 cells. (A, B) Cytotoxicity of PnL extract and piperine. 3T3-L1 cells were exposed to different concentrations of PnL extract (A) or piperine (B) for 72 h. Cytotoxicity was measured by MTT assays. (C, D) Oil Red O staining and measurement of lipid content. 3T3-L1 cells were differentiated and treated with DMSO, PnL extract (50 μg/mL), or piperine (50 μM). After differentiation for 10 days, cellular lipid droplets were stained with Oil Red O (upper plates are magnification ×10) (C) or eluted and measured at OD 500 nm (D). Data reflect the mean ± SD of three independent experiments. *, $p < 0.05$, and **, $p < 0.01$, compared to DMSO control.

RESULTS

***P. nigrum* Linne (PnL) Extract and Piperine Inhibit Lipid Accumulation in 3T3-L1 Cells.** Prior to determining the effect of PnL extract and its major constituent piperine on the lipid accumulation associated with adipocyte differentiation in 3T3-L1 cells, we determined the cytotoxicity of PnL extract and piperine using MTT assays. As shown in Figure 1, panels A and B, no significant effects of PnL extract and piperine on the proliferation of 3T3-L1 cells were observed for concentrations of 50 μg/mL and 200 μM, respectively. Subsequent Oil Red O staining indicated that PnL extract (50 μg/mL) and piperine (50 μM) significantly decreased lipid accumulation compared to the DMSO-treated control cells upon differentiation for 10 days (Figure 1C). To quantify the level of lipid accumulation, we extracted lipid from Oil Red O stained cells. The lipid contents in 3T3-L1 cells treated with PnL extract and piperine were reduced to 34.5 and 44.9% of control cells, respectively (Figure 1D).

PnL Extract and Piperine Down-Regulate the mRNA Expression of Adipogenic Genes. The effect of PnL extract and piperine on lipid deposition prompted us to investigate their roles in gene expression upon adipogenesis of 3T3-L1 cells. Real-time RT-PCR revealed that both treatments greatly decrease the mRNA level of adipogenic transcription factors PPARγ, SREBP-1c, and C/EBPβ (Figure 2A). The down-regulation of PPARγ mRNA level by the treatments led us to determine the expression of PPARγ target genes, adipsin, aP2, and LPL. Indeed, both PnL extract and piperine reduced the mRNA level of these genes significantly compared to DMSO control (Figure 2B). In all cases, the effect of piperine was more significant than the effect of the PnL extract.

Piperine Alleviates the Ligand-Induced Transcriptional Activity of PPARγ. PPARγ plays a pivotal role in adipocyte differentiation through regulation of adipogenic gene expression. To address the effect of piperine on the transcriptional activity of PPARγ, luciferase reporter gene assays were performed. HEK293 cells were cotransfected with PPRE-tk-luciferase reporter plasmid and PPARγ expression vector in the presence and absence of rosiglitazone, a specific ligand for PPARγ, and increasing concentrations of piperine. Subsequent luciferase assays indicated that piperine represses the transcriptional activity of PPARγ in both ligand-independent and -dependent manners (Figure 3). These results suggest that piperine may act as an antagonist of PPARγ, leading to inhibition of adipogenesis associated with PPARγ activation.

Piperine Inhibits the Agonist-Induced PPARγ Binding to Coactivator CBP. It has been well documented that PPARγ activation is achieved by its ligand (or agonist)-induced PPARγ binding to coactivators such as CREB-binding protein (CBP).[31,32] Thus, we postulated that the piperine-mediated PPARγ inactivation might result from the antagonistic activity of piperine on the ligand (rosiglitazone)-enhanced PPARγ binding to coactivator CBP. To confirm this hypothesis, the GST pull-down assay was employed using purified GST-CBP and His-PPARγ in the presence of rosiglitazone and piperine. As indicated in Figure 4A, rosiglitazone induced interaction between the N-terminal of CBP (amino acids 1−460) and the ligand-binding domain of PPARγ. This interaction was

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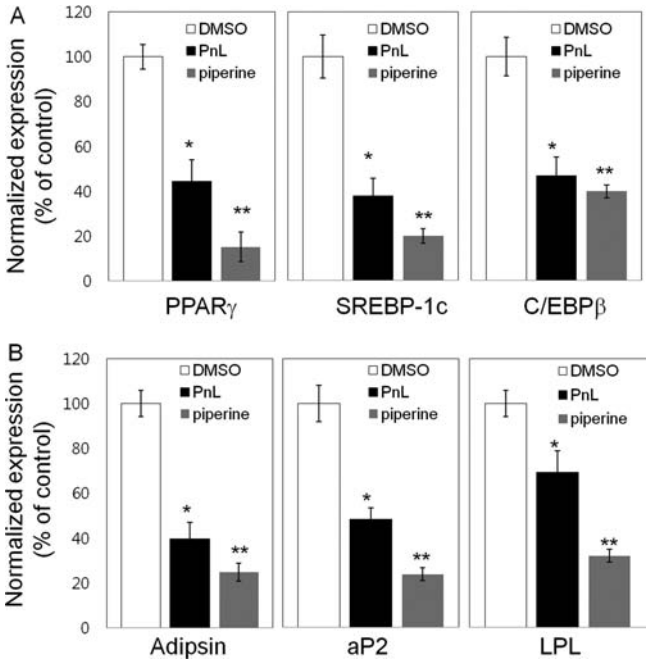

Figure 2. Effect of PnL extract and piperine on the expression of adipogenic genes. 3T3-L1 cells were differentiated and treated with DMSO, PnL extract (50 μg/mL), or piperine (50 μM). Ten days following differentiation of 3T3-L1, the mRNA expression of PPARγ, SREBP-1c, C/EBPβ, adipsin, aP2, and LPL was monitored by qRT-PCR. The mRNA expression is normalized using GAPDH and shown by percentage of DMSO control. Bars represent mean values ± SD of three independent experiments. *, $p < 0.05$, and **, $p < 0.01$, compared to DMSO control.

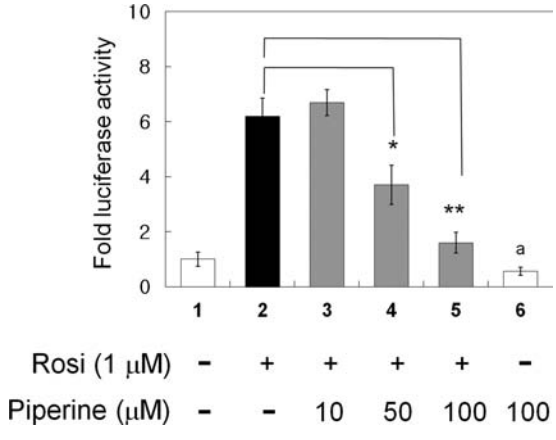

Figure 3. Piperine antagonizes PPARγ. HEK293 cells were transfected with vectors expressing PPARγ and PPRE-tk-luciferase reporter gene in the presence of rosiglitazone (Rosi, 1 μM) and increasing concentrations of piperine as indicated. Luciferase activity was measured as described under Materials and Methods. Luciferase values were normalized to the β-galactosidase activity. Upper shows the structure of reporter gene. Bars represent mean values ± SD of three independent experiments. *, $p < 0.05$, and **, $p < 0.01$, and a, $p < 0.05$, compared to DMSO control (lane 1).

gradually reduced by piperine in a dose-dependent manner, consistent with the result from the luciferase reporter assay. Gw9662, a known antagonist of PPARγ, was used for comparison (Figure 4B). Overall, our observations suggest that the piperine antagonism on PPARγ activity is attributed to its inhibitory effect on PPARγ binding to coactivator CBP.

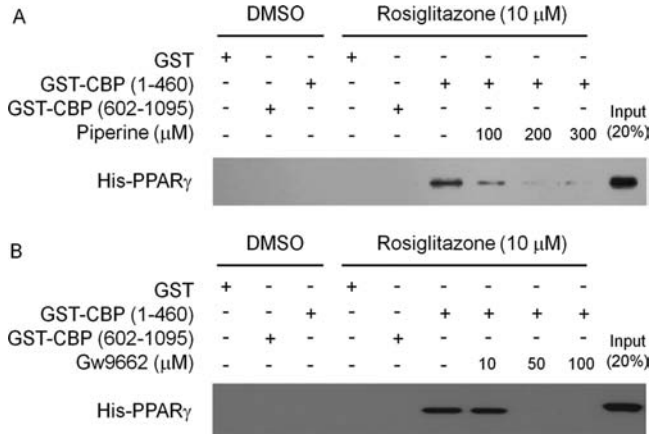

Figure 4. Piperine disrupts rosiglitazone-induced PPARγ binding to CBP. GST pull-down assays were performed as described under Materials and Methods. Immobilized GST-CBP (amino acids 1−460 or 602−1095) fusion protein was incubated with increasing concentrations of piperine (A) or GW9662 (B) in the presence of 1 μM rosiglitazone and His-tagged PPARγ ligand-binding domain. Western blotting with anti-His antibody shows the effect of piperine on CBP recruitment to rosiglitazone-bound PPARγ. Input represents 20% of PPARγ used for binding assays.

Microarray Analysis and Validation. To explore the role of piperine in genome-wide regulation of adipogenesis, we performed microarray analysis using piperine- or PnL extract-treated 3T3-L1 cells and Agilent Mouse 44k 4plex. We identified 5733 genes differentially expressed, >2-fold, by piperine and 7053 genes by PnL extract (Figure 5A). Among those regulated genes, 3398 genes were coregulated by piperine and PnL extract. Further clustering analysis emphasized that PnL extract shares with piperine in controlling gene expression (Figure 5B). Both treatments significantly reduced mRNA expression levels of genes associated with adipocyte differentiation (Table 2). Consistent with our RT-qPCR data shown in Figure 2, the mRNA expression of PPARγ, SREBP-1c, and C/EBPβ, adipsin, aP2, and LPL was significantly reduced. Other down-regulated genes were adiponectin, Lpin1, resistin, Scd1, and perilipin. In addition, genes associated with lipid biosynthesis and transport were greatly repressed, whereas lipolysis-associated genes were up-regulated by either treatment (Table 3). Some of the regulated genes newly identified from microarray analysis were validated by RT-qPCR: FATCD36, GLUT4, SCD1, and C/EBPβ (Figure 5C). Overall, our RT-qPCR data were fairly consistent with microarray data, with little difference in magnitude. These genome-wide studies strongly suggest that piperine, as a critical component of PnL extract, plays a common role with PnL extract in regulating lipid metabolism.

DISCUSSION

Obese condition increases the risk of fat-related disorders such as hyperglycemia, hyperlipidemia, hypercholesterolemia, and diabetes. There are many reports that natural compounds from herbal medicines or fruits have efficacies in disease-associated metabolic disorder and lipid metabolism.[34−36] The fruit of *P. nigrum* has been used in oriental herbal medicine and worldwide as a spice in the form of black pepper. Piperine, one of the natural alkaloids, is a pungent ingredient of black pepper. In recent decades, various clinical efficacies of piperine have been reported.[15] Focusing on its roles in lipid metabolism,

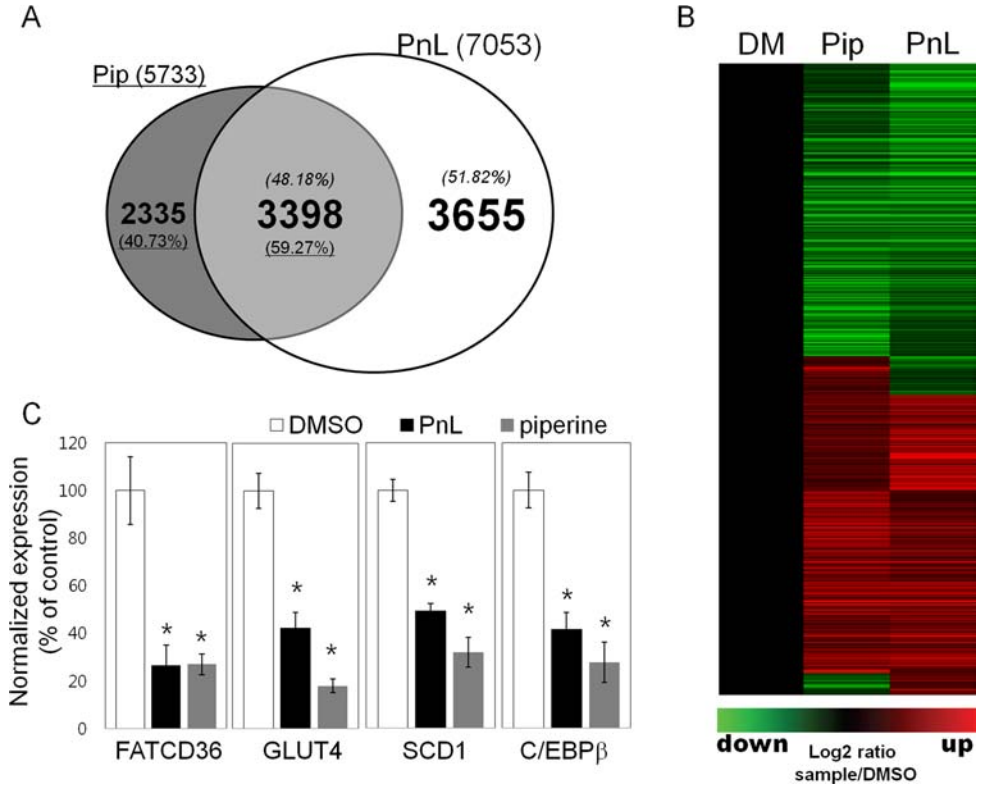

Figure 5. Gene expression profilings of PnL extract- and piperine-regulated adipogenesis and validation in 3T3-L1 cells. (A) Genes expressed differentially during adipogenesis by piperine or PnL ext are indicated in a Venn diagram. (B) Clustering analysis of 9388 genes was regulated by PnL extract and piperine. (C) Validation of gene expression selected in piperine- or PnL extract-regulated adipogenesis. Bars represent mean values ± SD of three independent experiments. *, $p < 0.001$, vs DMSO control.

Table 2. mRNA Expression Level of Genes Associated with Fat Cell Differentiation

			fold change[a]	
name	gene description	GenBank	Pip	PnL
Slc2a4	solute carrier family 2, member 4	NM_009204	0.073	0.049
Cebpb	CCAAT/enhancer binding protein (C/EBP), β	NM_009883	0.400	0.399
Cebpa	CCAAT/enhancer binding protein (C/EBP), α	NM_007678	0.256	0.206
Srebf1	sterol regulatory element binding factor 1	NM_011480	0.527	0.444
Pparg	peroxisome proliferator activated receptor γ	NM_011146	0.178	0.266
Adipoq	adiponectin, C1Q and collagen domain containing	NM_009605	0.355	0.095
Adn	complement factor D (adipsin) (Cfd)	NM_013459	0.111	0.026
Lxra	nuclear receptor subfamily 1, group H, member 3	NM_013839	0.395	0.416
Ncor2	nuclear receptor corepressor 2	NM_011424	0.744	0.446
Fabp4	fatty acid binding protein 4, adipocyte (aP2)	NM_024406	0.216	0.440
Lpin1	lipin 1	NM_015763	0.123	0.219
Retn	resistin	NM_022984	0.054	0.028
Scd1	stearoyl-coenzyme A desaturase 1	NM_009127	0.090	0.054
Plin	perilipin	NM_175640	0.133	0.089

[a]Fold change indicates the fold change of mRNA expression level compared to untreated sample.

piperine inhibits the activity of acyl-CoA:cholesterol acyltransferase (ACAT), which is responsible for cholesteryl ester (CE) synthesis, thus leading to a reduction of lipid droplets in macrophages.[37] Other studies have suggested that piperine improves hyperglycemia, hyperlipidemia, and hypercholesterolemia by reducing the level of blood lipid, glucose, and cholesterol.[27,28,38] In our studies, we found that PnL extract and its constituent piperine reduce lipid storage in 3T3-L1 cells and block their differentiation into adipocytes.

Fully differentiated adipocytes increase the expression of adipocyte-specific genes. PnL extract and piperine reduced the mRNA expression level of PPARγ, SREBP-1c, and C/EBPβ, key transcription factors associated with adipogenesis. The downstream target genes of these factors such as adipsin, aP2 (or Fabp4), and LPL were also down-regulated by PnL extract or piperine treatment in differentiated 3T3-L1 cells. As shown in our microarray data (Tables 2 and 3), the expression of other genes was also attenuated by both treatments: fatty acid translocase CD36, apolipoprotein C−II (Apoc2), and keratinocyte lipid-binding protein Fabp5 associated with fatty acid uptake and transport; Fasn, Scd1, Acsl1, Fads3, and Dgat1 involved in lipid synthesis; lipid droplet-associated protein

Table 3. mRNA Expression Level of Genes Associated with Lipid Metabolism

name	gene description	GenBank	fold change[a] Pip	fold change[a] PnL
Lipid Biosynthetic Process				
Fasn	fatty acid synthase	NM_007988	0.336	0.151
Acsl1	acyl-CoA synthetase long-chain family member 1	NM_007981	0.152	0.070
Acsl5	acyl-CoA synthetase long-chain family member 5	NM_027976	0.360	0.394
Scd1	stearoyl-coenzyme A desaturase 1	NM_009127	0.090	0.054
Fads3	fatty acid desaturase 3	NM_021890	0.439	0.687
Cyp51	cytochrome P450, family 51	NM_020010	0.234	0.396
Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)	NM_010271	0.279	0.041
Dgat2	diacylglycerol *O*-acyltransferase 2	NM_026384	0.144	0.248
Dgat1	diacylglycerol *O*-acyltransferase 1	NM_010046	0.220	0.371
Adipor1	adiponectin receptor 1	NM_028320	0.641	0.564
Adipor2	adiponectin receptor 2	NM_197985	0.250	0.402
Lipid Catabolic Process				
Pld4	phospholipase D family, member 4	NM_178911	2.863	4.126
Plcg1	phosphoinositide phospholipase C-γ-1 (ELP)	AF027185	3.446	3.825
Clps	colipase, pancreatic	NM_025469	2.516	2.510
Plcd1	phospholipase C, δ 1	NM_019676	2.717	2.399
Acot7	acyl-CoA thioesterase 7	NM_133348	1.890	2.256
Pla2g2d	phospholipase A2, group IID	NM_011109	2.606	2.178
Hexa	hexosaminidase A	NM_010421	4.806	1.987
Oc90	otoconin 90	NM_010953	1.933	1.928
Cel	carboxyl ester lipase	Nm_009885	1.524	2.443
Uptake and Transport				
Slc37a4	solute carrier family 37, member 4	NM_008063	0.615	0.276
Slc27a4	solute carrier family 27, member 4	NM_011989	0.353	0.620
Slc22a4	solute carrier family 22, member 4	NM_019687	0.452	0.231
Cd36	CD36 antigen (Fatcd36)	NM_007643	0.187	0.171
Lpl	lipoprotein lipase	NM_008509	0.230	0.279
Apoc2	apolipoprotein C-II	NM_009695	0.341	0.173
Fabp4	fatty acid binding protein 4, adipocyte (ap2)	NM_024406	0.216	0.440
Fabp5	Mal1 mrna for keratinocyte lipid-binding protein	X70100	0.105	0.143
Slco2a1	prostaglandin transporter PGT mrna, complete cds	AF323958	8.903	9.450

[a]Fold change indicates the fold change of mRNA expression level compared to untreated sample.

perilipin; PPARγ coactivator lipin 1; adiponectin and adiponectin receptors 1 and 2; adipokines-associated insulin sensitivity; GLUT4 involved in glucose uptake; and liver X receptor LXRα. In contrast, PnL extract and piperine increased the mRNA expression level of lipid catabolic process-associated genes, such as Pld4, Plcg1, colipase, plcd1, Acot7, and Cel (Table 3). Overall, these results suggest that PnL extract and piperine may inhibit adipogenesis of 3T3-L1 preadipocyte by down-regulating many genes associated with lipid accumulation in 3T3-L1 cells during differentiation and by up-regulating various genes associated with lipid catabolic process. In most cases, PnL extract and piperine showed similar regulation patterns of gene expression. Given that piperine is a major component of PnL extract,[20] our data suggest that piperine is a functional ingredient of the PnL extract in playing a role in lipid metabolism. However, other components in PnL extract may account for differences in the magnitude of gene expression, lipid accumulation, and cellular toxicity.

It has been reported that PPARγ plays a pivotal role in adipocyte differentiation through regulation of adipogenic gene expression.[7,8] As judged from the expression profiling of genes down-regulated by piperine, we found that the most genes are downstream targets of PPARγ. These observations prompted us to investigate the molecular mechanism played by piperine. Our luciferase reporter assay indicated that piperine significantly inhibits the PPARγ transcriptional activity. Subsequent binding assays displayed that piperine, like the known antagonist Gw9662, disrupts the ligand-dependent interaction between PPARγ and CBP. It has been well documented that CBP functions as a PPARγ coactivator by associating with agonist-bound PPARγ.[31,32] In addition, CBP is indispensible for adipogenesis through the activation of PPARγ.[39] Together with a positive role of PPARγ in adipogenesis, we suggest that piperine may inhibit adipocyte differentiation of 3T3-L1 cells by its antagonistic activity against PPARγ. Computer-based structural analysis implied that the ligand-binding domain of PPARγ can accommodate piperine in its binding pocket (data not shown), further supporting that piperine may function as an antagonist of PPARγ, thus disrupting the interaction between agonist-bound PPARγ and coactivator CBP. During our studies, we also found that piperine represses the transcriptional activity and expression level of LXRα (data not shown), another transcriptional factor that plays a pivotal role in inducing adipogenesis and biosynthesis of cholesterol and fatty acid through up-regulation of various genes such as SREBP-1c, Fasn, Scd1, and LPL. Interestingly, LXRα is not only up-regulated by PPARγ[40−43] but also increases the expression of SERBP-1c[44,45] and PPARγ,[46] sharing several target genes such as Fasn, Scd1, LPL, and GLUT4. Therefore, these positive feedback loops may result in cascade regulation of lipid metabolism. Taken together, our findings suggest that piperine, a major component of black pepper, inhibits fat cell differentiation by down-regulating the transcriptional activity of PPARγ (and LXRα) and suppressing the expression of PPARγ (and LXRα), thus leading to its potential use in the treatment of obesity-related diseases.

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Notes

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