Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 22 (2011) 910–920

Journal of Nutritional Biochemistry

Curcumin inhibits adipocyte differentiation through modulation of mitotic clonal expansion☆

Choon Young Kim[a], Thuc T. Le[b], Chihyu Chen[a], Ji-Xin Cheng[b,c], Kee-Hong Kim[a,*]

[a]Department of Food Science, Purdue University, West Lafayette, IN 47907, USA
[b]Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907, USA
[c]Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

Received 26 January 2010; received in revised form 21 July 2010; accepted 2 August 2010

Abstract

Adipocyte differentiation is a key process in determining the number of mature adipocytes in the development of obesity. Here, we examined the function of curcumin, a dietary polyphenol found in turmeric, and its underlying mechanisms in adipocyte differentiation. Our study reveals that curcumin exerts an anti-adipogenic function both in 3T3-L1 murine cells and in human primary preadipocytes as determined by intracellular lipid accumulation assay, quantitative analysis of adipocyte marker gene expression and a noninvasive multimodal Coherent Anti-Stokes Raman Scattering (CARS) microscopic analysis of intracellular curcumin. The inhibitory action of curcumin was largely limited to the early stage of adipocyte differentiation, where curcumin was found to inhibit mitotic clonal expansion (MCE) process as evidenced by impaired proliferation, cell-cycle entry into S phase and the S to G2/M phase transition of confluent cells, and levels of cell cycle-regulating proteins with no significant effect on cell viability and cytotoxicity. This, in turn, resulted in inhibition of mRNA levels of early adipogenic transcription factors, particularly Krüppel-like factor 5 (KLF5), CCAAT/enhancer binding proteinα (C/EBPα) and peroxisome proliferator-activated receptorγ (PPARγ), in the early stage of adipocyte differentiation. Supplementation with rosiglitazone, a PPARγ ligand, during the early stage of adipocyte differentiation partially rescued curcumin-inhibited adipocyte differentiation. Collectively, our results show that curcumin is an anti-adipogenic dietary bioactive component largely involved in the modulation of the MCE process during the early stage of adipocyte differentiation.

Keywords: Curcumin; 3T3-L1; Adipogenesis; Mitotic clonal expansion; Coherent Anti-Stokes Raman Scattering microscope

1. Introduction

Obesity-associated chronic diseases such as type 2 diabetes and cardiovascular disease have been shown to contribute to a rising incidence of mortality and morbidity [1]. Both an increased number of adipocytes (hyperplasia) due to enhanced differentiation of pre-adipocytes to adipocytes, and an increased size of adipocytes (hypertrophy) due to lipid accumulation are shown to participate in the expansion of adipose tissue. A recent human study suggests that tight regulation of adipocyte number is the key determinant for adipose mass not only in childhood and adolescence, but also in adulthood [2]. This finding supports the idea of adipocyte hyperplasia as an effective target for treating or preventing the development of obesity and its related diseases.

The molecular mechanisms underlying hormonal and nutritional regulation of adipocyte differentiation have been extensively studied using *in vitro* models of mouse preadipocyte cell lines such as 3T3-L1 and 3T3-F442A cells [3,4]. In response to a standard adipogenic cocktail, including insulin, glucocorticoid and an inhibitor of cyclic AMP phosphodiesterase, growth-arrested confluent preadipocytes are known to reenter the cell cycle for an additional two rounds of division, known as mitotic clonal expansion (MCE). Within 24 h after induction by the adipogenic cocktail, activated MCE concomitantly initiates a well-programmed series of transcriptional activation with expression of early adipogenic transcription factors such as Krüppel-like factor 5 (KLF5) and CCAAT/enhancer binding proteinβ (C/EBPβ), followed by C/EBPδ, PPARγ and C/EBPα, which are involved in changing mature adipocyte phenotype [4]. By 48 h of the indiction of adipocyte differentiation, the cells complete this coordinated activation of MCE and early adipogenic transcriptional program, and begin to express late markers of adipocyte differentiation, including genes encoding adipokines such as leptin, adiponectin and resistin.

Despite continuous attention on dietary phytochemicals to act as rich therapeutic and/or preventive sources for many diseases, few such compounds have been known to have a direct role in adipocyte

☆ This work was supported in part by the Ralph W. and Grace M. Showalter Research Trust and by the Agriculture and Food Research Initiative (AFRI) Grant 2009-65200-05994 from the USDA National Institute for Food and Agriculture to KHK, and a NIH R0 grant EB007243 to JXC. CYK is supported by the Agricultural Research Assistantship Program from Purdue University. TTL is supported by a NIH Ruth L. Kirschstein NRSA postdoctoral fellowship.

* Corresponding author. Tel.: +1 765 496 2330; fax: +1 765 494 7953.
E-mail address: keehong@purdue.edu (K.-H. Kim).

0955-2863/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2010.08.003

differentiation, thereby serving as a means to control adipose tissue mass. Curcumin (diferuloylmethane) is the yellow pigment and the key bioactive compound found in the rhizome of the perennial herb turmeric (*Curcuma longa*), a dietary spice commonly used in curry [5–7]. Curcumin is known to have anti-oxidative, anti-inflammatory and anti-carcinogenic properties [5–7]. The beneficial role of curcumin in various diseases, such as cancer, psoriasis, heart failure and Alzheimer's disease, has been well established [8–12]. In addition, a number of human studies proved that curcumin has no significant toxic effects at very high doses (8 g/day), suggesting curcumin as a safe and effective dietary compound in humans. Despite the fact that obesity is associated with activated oxidative stress and inflammation in adipose tissue, the beneficial role of curcumin and its underlying mechanisms in the development of adipose tissue are still under investigation. Recently, curcumin has been shown to ameliorate obesity-induced inflammation in adipose tissue and the liver with improved glycemic control and a slight decreased body weight gain in obese animals [13,14]. In these studies, a preventive role of curcumin in the development of high-fat diet-induced obesity in animals has been proposed [14,15]. Ejaz et al. [15] also demonstrated a potential inhibitory function of curcumin in adipocyte differentiation. These findings suggest that curcumin could be effective in modulating generation of new adipocytes. However, the molecular mechanisms underlying curcumin-regulated adipocyte differentiation is unclear.

Using cellular/biochemical techniques and an imaging tool, here we demonstrate that curcumin inhibits murine and human adipocyte differentiation with little effect on viability of differentiating cells. We elucidated that this anti-adipogenic function of curcumin is largely limited to the early stage of adipocyte differentiation, where curcumin inhibits the proliferation and cell-cycle progression of postconfluent preadipocytes in MCE with a subsequent suppression of pro-adipogenic transcription factors, KLF5, PPARγ and C/EBPα. We also showed that rosiglitazone, a PPARγ agonist, is able to partially rescue the inhibitory effect of curcumin on adipocyte differentiation. Together, our results elucidate that MCE process plays a critical role in mediating the anti-adipogenic function of curcumin.

2. Materials and methods

2.1. Materials and reagents

Curcumin, dexamethasone (DEX), 3-isobutyl-1-methyl-xanthine (IBMX) and propidium iodide solution were purchased from Sigma. Fetal calf serum (FCS) and fetal bovine serum (FBS) were purchased from PAA Cell Culture Company. Rosiglitazone was purchased from Calbiochem. Dulbecco's Modified Eagle's Medium (DMEM) and 0.25% trypsin-EDTA were purchased from Thermo Scientific. Medium (preadipocyte medium, adipocyte differentiation medium and adipocyte maintenance medium) related to subcutaneous human adipocyte culture was obtained from ZenBio, Inc.

2.2. Cell culture and treatment conditions

3T3-L1 preadipocytes obtained from American Type Culture Collection were cultured in DMEM containing 10% (v/v) FCS. Two-day postconfluent 3T3-L1 preadipocytes, designated to Day 0, were differentiated with DMEM supplemented with 10% FBS and an adipogenic cocktail containing 1 μg/ml insulin, 0.5 mM IBMX and 5 μM DEX for 2 days. Cells were then maintained in 10% FBS-DMEM with insulin for another 2 days, followed by an additional 2 days of culture with DMEM containing 10% FBS, at which time >90% of cells were differentiated into mature adipocytes. All medium contained 100 U/ml penicillin, 100 μg/ml streptomycin and 0.11 g/L sodium pyruvate. A subcutaneous human adipocyte cell line was purchased from ZenBio. Cell culture process was followed by manufacturer's protocol. Briefly, subcutaneous human preadipocytes were maintained in preadipocyte medium. Differentiation of confluent subcutaneous human preadipocytes was initiated by adding fresh adipocyte differentiation medium. After 7 days, medium was replaced with fresh adipocyte maintenance medium. Adipocyte differentiation was continued until Day 14 when lipid droplets were clearly observed. All cells were maintained at 37°C in a humidified 5% CO_2 atmosphere. Oil red O (ORO) staining was performed on Day 6 to stain the accumulated lipid droplets in differentiated adipocytes [16]. The ORO-stained cells were photographed. Spectrophotometric quantification of the ORO-stained lipid was performed by measuring optical density at 490 nm after extracting the ORO dye from cells by isopropanol.

2.3. Isolation of total RNA and real-time reverse transcription-PCR

By following manufacturer's instruction, total RNA in 3T3-L1 cells was extracted using Trizol Reagent (Invitrogen) and 1 μg/μl of isolated RNA was subjected to the reverse transcription reaction using SuperScript II kit (Invitrogen) for cDNA synthesis. Generated cDNA was subjected to real-time reverse transcription (RT)-PCR reactions using thermocyclers from Applied Biosystems or Stratagene. In real-time RT-PCR, diluted cDNA was added to a SYBR premixed Taq reaction mixture containing 100 ng/ml PCR primers. The sequence of primers corresponding to mouse adipogenic genes used in Fig. 1C is as follows: C/EBPβ (forward, 5′-AGC GGC TGC AGA AGA AGG T-3′; reverse, 5′-GGC AGC TGC TTG AAC AAG TTC-3′); PPARγ (forward, 5′-CCC AAT GGT TGC TGA TTA CAA AT-3′; reverse, 5′-TTT CTA CTT TGA TCG CAC TTT GGT ATT CT-3′); C/EBPα (forward, 5′-GGT TTA GGG ATG TTT GGG TTT TT-3′; reverse, 5′-AAG CCC ACT TCA TTT CAT TGG T-3′); adiponectin (forward, 5′-GAT GCA GGT CTT CTT GGT CCT AA-3′; reverse, 5′-GGC CCT TCA GCT CCT GTC A-3′); leptin (forward, 5′-CAC ACA CGC AGT CGG TAT CC-3′; reverse, 5′-AGC CCA GGA ATG AAG TCC AA-3′); resistin (forward, 5′-TGC CAG TGT GCA AGG ATA GAC T-3′; reverse, 5′-CGC TCA CTT CCC CGA CAT-3′); β-actin (forward, 5′-AGA TGA CCC AGA TCA TGT TTG AGA-3′; reverse, 5′-CAC AGC CTG GAT GGC TAC GT-3′). The sequences of primers used in Fig. 5 and 6 are as follows: C/EBPβ (forward, 5′-GCA AGA GCC GCG ACA AG-3′; reverse, 5′-GGC TCG GGC AGC TGC TT-3′); KLF5 (forward, 5′-CCG GAG ACG ATC TGA AAC AC-3′; reverse, 5′-GGA GCT GAG GGG TCA GAT ACT T-3′); PPARγ (forward, 5′-CCA CCA ACT TCG GAA TCA GCT-3′; reverse, 5′-TTT GTG GAT CCG GCA GTT AAG A-3′); C/EBPα for real-time PCR (forward, 5′-GCT GGA GTT GAC CAG TGA CA-3′; reverse, 5′-AAA CCA TCC TCT GGG TCT CC-3′); β-actin (forward, 5′-TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA-3′; reverse, 5′-CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG-3′).

2.4. Multimodal Coherent Anti-Stokes Raman Scattering microscope

A multimodal microscope capable of simultaneous Coherent Anti-Stokes Raman Scattering (CARS) and two-photon excitation fluorescence (TPEF) imaging has been previously described [17–19]. Briefly, for CARS imaging of lipid droplets in adipocytes, the wave number difference between the pump laser and Stokes laser tuned to 2840 per centimeter, which matches the Raman shift of the symmetric CH_2 stretch vibration in lipid molecules. The forward detected CARS signal was collected by an air condenser (numerical aperture=0.55), transmitted through a 600/65-nm bandpass filter and detected by a photomultiplier tube (PMT, H7422-40, Hamamatsu, Japan). For TPEF imaging of intracellular fluorescent curcumin, the back-reflected signal was collected by the objective, spectrally separated from the excitation source, transmitted through a 520/40-nm bandpass filter and detected by a PMT (H7422-40, Hamamatsu) mounted at the back port of the microscope. Acquisition time for each image was 1.12 s. Images were analyzed using Fluo-View software (Olympus).

2.5. Flow cytometric analysis

A Cell Lab Quanta SC flow cytometer (Beckman Coulter, Brea, CA, USA) was used to assess the effect of curcumin on cell-cycle progression of differentiating 3T3-L1 cells. For this experiment, 3T3-L1 preadipocytes stimulated by the adipogenic cocktail in the absence or presence of 20 or 30 μM curcumin for the indicated time points were employed in this experiment. Cell pellets harvested at each time point were fixed with 70% ethanol for at least 2 h on ice and washed twice with PBS. After staining with 40 μg/ml propidium iodine solution containing 500 μg/ml of RNase A for 30 min at 37°C, cells were subjected to cell-cycle analysis using a flow cytometer.

2.6. Cell viability, proliferation, cytotoxicity and bromodeoxyuridine incorporation assays

A 3-(4,5-dimethyl-thiazol-yl-2)-2,5-diphenyl tetrazolium bromide (MTT) reduction was determined to examine the effect of curcumin on cell viability. Briefly, following the treatment of cells with different concentrations of curcumin in 10% FCS- or FBS-containing DMEM for 24 or 48 h, cells were incubated with MTT solution (0.5 mg/ml) for 1 h at 37°C. The formation of a violet precipitate, formazan, was monitored at a wavelength of 595 nm and with a microplate reader (Beckman Coulter). Cytotoxicity was measured based on lactate dehydrogenase (LDH) activity in cell culture medium. Cytotoxicity assay was performed using the CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Briefly, medium were collected when cells were cultured with curcumin for 24 and 48 h. Medium was incubated with reconstituted substrate mix for 30 min at room temperature and then the reaction was stopped by stop solution. LDH activity was measured at 490 nm by a microplate reader. 3T3-L1 cell proliferation was determined by counting the cells with a hemocytometer. Cell counting was determined in triplicate wells in each condition, and the value for each well was the mean of the cell count in four different fields of the hemocytometer. The bromodeoxyuridine (BrdU) incorporation assay was also employed to investigate the influence of curcumin on cell proliferation using a commercially available BrdU cell proliferation assay kit (Calbiochem, Germany). Briefly, 3T3-L1 cells treated with different concentrations of curcumin in 10% FBS-containing DMEM medium for 24 or 48 h in a 96-well plate were

labeled with BrdU for 18 h. After BrdU labeling, medium was removed and incorporated BrdU in the cell was monitored using an antibody generated against BrdU. BrdU incorporation was measured at a wavelength of 450 nm and at 595 nm for background adjustment with a microplate reader.

2.7. Immunoblot assay

Two-day confluent 3T3-L1 cells were differentiation by treatment with the standard differentiation cocktail in the absence or presence of curcumin (0, 10, 20 and 30 μM). Cells were harvested by scrapping at Days 0, 1 and 2. Cell pellets were prepared by cell lysis buffer containing Tris-Cl (100 mM, pH 7.5–8), NaCl (100 mM), 0.5% TritonX-100, protease inhibitor cocktail, sodium orthovanadate (1 mM) and sodium fluoride (10 mM). The protein concentration was determined using the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA). Proteins were separated by 12% SDS-PAGE and transferred to nylon membrane. Immunoblot was performed by phospho-C/EBPβ (Thr235) (Cell Signaling, Danvers, MA, USA), C/EBPβ (C-19) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), cyclin A (Sigma, St. Louis, MO, USA) and cdk2 (MBL, Japan) antibodies at 4°C overnight and then visualized with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) and an enhanced chemiluminescence kit (Pierce, Rockford, IL, USA).

2.8. Statistical analysis

Data are shown as means±S.E.M. Statistical analysis was performed using SAS 9.0 software. One-way ANOVA was used to determine the significance of treatment effect and interactions. Significant differences between group means were assessed by Dunnette's multiple comparison. *P* values lower than .05 were regarded as statistically significant.

3. Results

3.1. Curcumin inhibits differentiation of 3T3-L1 preadipocytes in a dose-dependent manner

To elucidate the function of curcumin and its underlying molecular mechanism in adipocyte differentiation, we first tested the effect of curcumin on the differentiation of 3T3-L1 preadipocytes. Treatment of differentiating 3T3-L1 preadipocytes with increasing concentrations of curcumin for 6 days resulted in a dose-dependent inhibition of adipocyte differentiation as judged by ORO staining (Fig. 1A). Quantification of ORO-stained intracellular lipid droplets allowed us to calculate the half maximal inhibitory concentration (IC_{50}) of curcumin to approximately 16.1 μM, with complete inhibition of adipocyte differentiation at 30 μM curcumin (Fig. 1B). Furthermore, mRNA levels of adipocyte marker genes such as C/EBPβ, PPARγ, C/EBPα, leptin, adiponectin and resistin were lower in curcumin-treated 3T3-L1 cells for 6 days (Fig. 1C). To examine whether curcumin's anti-adipogenic function could also be seen in non-murine cells, the effect of curcumin on differentiation of primary human preadipocytes was tested. Primary human preadipocytes were subjected to adipocyte differentiation for 14 days in the presence or absence of curcumin (0–12.5 μM) in accordance with the protocol as detailed in the Materials and Methods section. After 14 days of adipocyte differentiation, the intracellular lipid droplet accumulation in these human adipocytes was assessed by ORO staining. Treatment of differentiating primary human preadipocytes with various concentrations of curcumin resulted in a dose-dependent inhibition of adipocyte differentiation (Fig. 1D) with a maximum 80% inhibition at 12.5 μM curcumin (Fig. 1D). Collectively, these results demonstrate the anti-adipogenic property of curcumin *in vitro*.

3.2. The early stage of differentiation is critical for the inhibitory role of curcumin in adipocyte differentiation

3T3-L1 adipocyte differentiation initiated by an exposure to the adipogenic cocktail consists of three distinct stages, including the early stage (Days 0–2), the postmitotic intermediate stage (Days 3–4) and the terminal stage (after Day 4) [3]. To elucidate the molecular basis underlying curcumin-inhibited adipocyte differentiation, we attempted to identify the critical stage of adipocyte differentiation which is specifically affected by curcumin treatment. Differentiating 3T3-L1 cells exposed to the adipogenic cocktail were treated with 30 μM curcumin at various time points during adipocyte differentiation as illustrated in Fig. 2A. After 6 days of differentiation, the levels of adipocyte differentiation of these cells were determined by qualitative (Fig. 2B) and quantitative (Fig. 2C) analysis of ORO-stained intracellular lipid droplets. As expected, 3T3-L1 cells treated with 30 μM curcumin for 6 days showed a complete inhibition of adipocyte differentiation (i.e., Treatment 3 in Fig. 2C). Similar with this, cells incubated with curcumin during treatments from 4 to 6 exhibited significantly reduced levels of intracellular lipid droplet. However, differentiating 3T3-L1 cells treated with curcumin only in Day 1 (Treatment 7), Days 2–4 (Treatments 10 and 11), Days 2–6 (Treatments 8 and 9) or Days 4–6 (Treatment 12) of adipocyte differentiation resulted in a moderate inhibition of adipocyte differentiation to 20–40% compared with control cells (Treatment 2 in Fig. 2C). These results suggest that complete inhibition of adipocyte differentiation by curcumin is largely limited to the early stage (i.e., Days 0–2) and, to a lesser extent, postmitotic stages of adipocyte differentiation.

To correlate between the anti-adipogenic function and the presence of curcumin in adipocytes, we next monitored the absorption property of curcumin in differentiated 3T3-L1 cells incubated with curcumin using simultaneous CARS and TPEF imaging analysis. CARS microscopy is a noninvasive and label-free imaging technique, which is highly sensitive for optical examination of lipid-rich biological structures both *in vitro* and *in vivo* [20–23]. Since curcumin is naturally fluorescent in the visible green spectrum, we

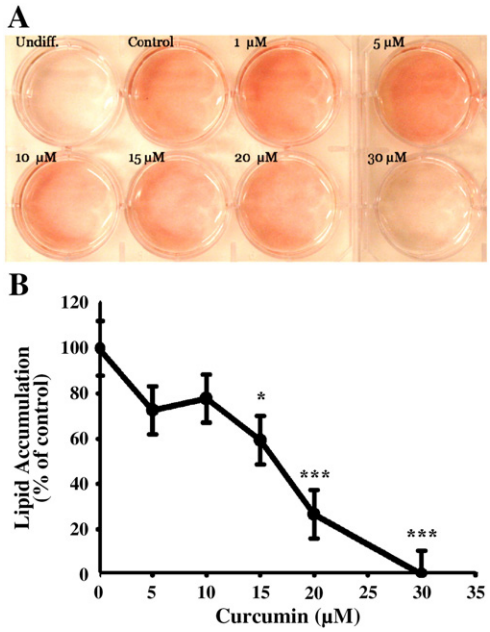

Fig. 1. Curcumin inhibits adipocyte differentiation in a dose-dependent manner. Two-day postconfluent 3T3-L1 preadipocytes were subjected to adipocyte differentiation for 6 days in the presence of dimethyl sulfoxide (DMSO) or various concentrations (0–30 μM) of curcumin in DMSO. (A) After 6 days of treatment of 3T3-L1 cells with curcumin, cells were subjected to ORO staining. (B) These cells were then subjected to quantitative analysis of intracellular lipid accumulation. (C) Adipocyte markers (C/EBPβ, PPARγ, C/EBPα, leptin, adiponectin and resistin) were analyzed by real-time PCR using total RNA isolated from curcumin-treated 3T3-L1 cells. The signals were normalized to β-actin internal control, and the results were expressed as relative fold of induction. (D) Primary human preadipocytes were subjected to adipocyte differentiation for 14 days in the presence of DMSO or various concentrations (0–12.5 μM) of curcumin in DMSO. After 14 days of cell differentiation, cells were subjected to ORO staining and quantitative analysis of ORO-stained intracellular lipids. A representative ORO-stained image is shown. Data are presented as means±S.E.M. ($^{*}P<.05$; $^{***}P<.001$), $n=3$–6.

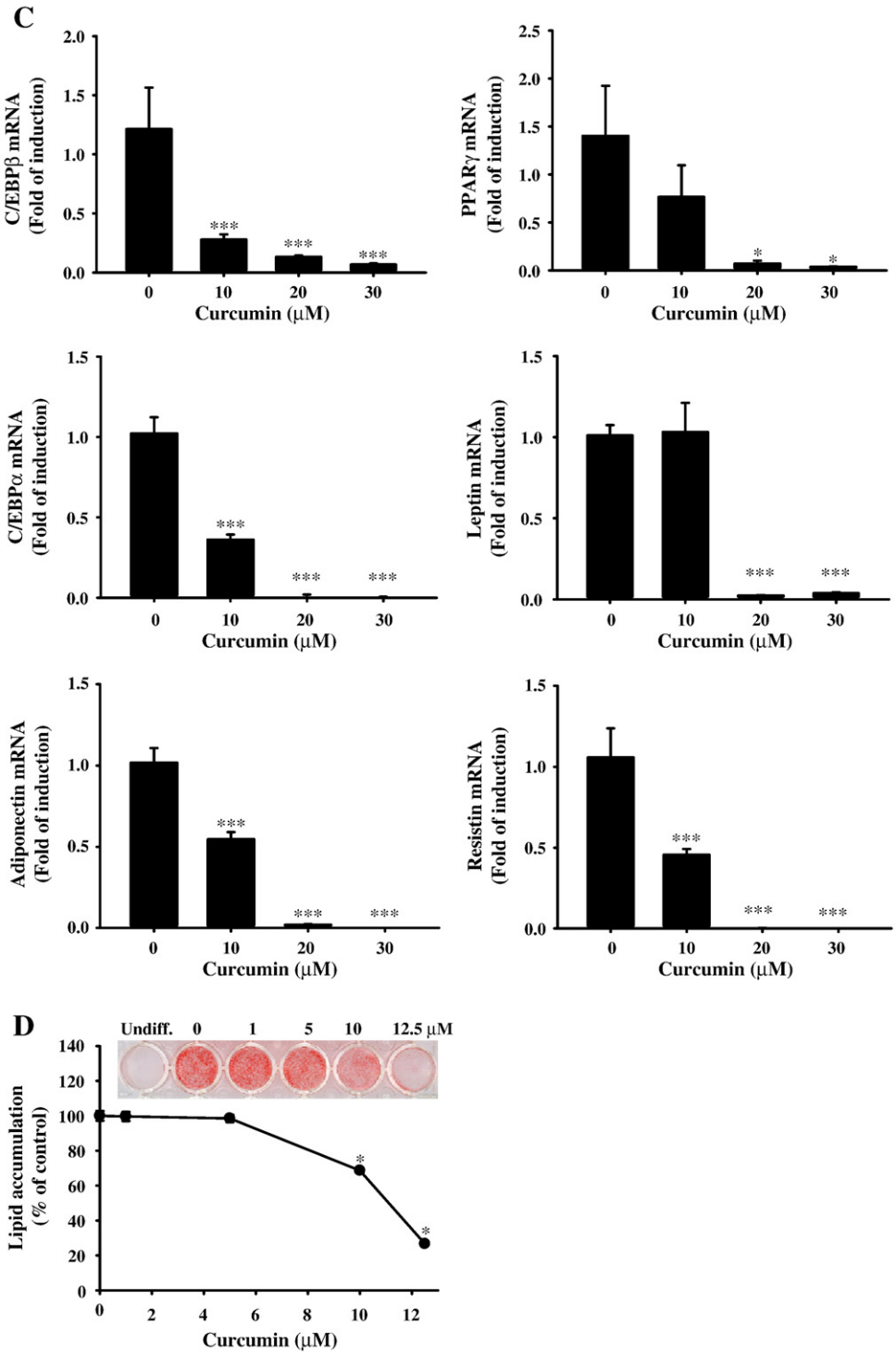

Fig. 1 (*continued*).

performed simultaneous imaging of lipid droplet accumulation with CARS and intracellular curcumin with TPFE in differentiating 3T3-L1 cells using a multimodal CARS microscope. As shown in Fig. 2D, control adipocytes differentiated for 6 days exhibited accumulation of various sizes of lipid droplets detected by CARS microscope. Similar with the results shown in Fig. 2B and C, CARS microscopy also revealed that differentiating 3T3-L1 cells incubated with either 20 or 30 μM curcumin only in the early stage of differentiation resulted in a significant reduction in both the number and size of intracellular lipid droplets as well as in a sustained green fluorescent signal of curcumin after 6 days of differentiation (Fig. 2D).

3.3. Curcumin inhibits the MCE process in the early stage of adipocyte differentiation

A number of studies demonstrated a cytotoxic and/or pro-apoptotic property of curcumin in various cell lines [24–26]. These suggest that the anti-adipogenic role of curcumin found in this study

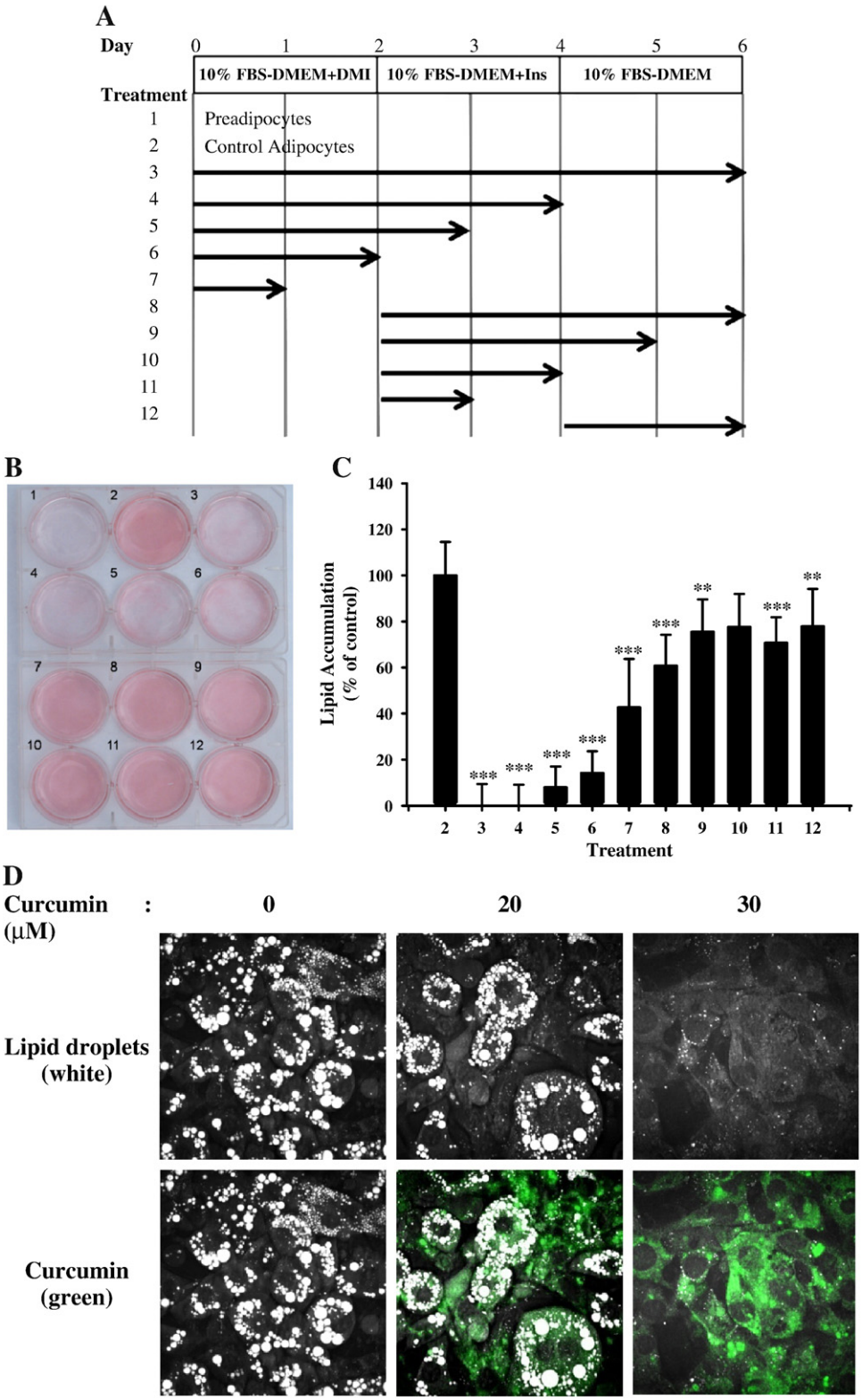

Fig. 2. The inhibitory effect of curcumin occurs mainly in the early stage of differentiation. Two-day postconfluent 3T3-L1 preadipocytes were subjected to adipocyte differentiation for 6 days with 30 μM curcumin at the indicated time points, as shown in (A). After these treatments, cells were subjected to ORO staining (B) and quantitative analysis of ORO-stained intracellular lipids (C). (D) Differentiating 3T3-L1 cells for 6 days treated with curcumin (0, 20 and 30 μM) only during Day 0 to Day 2 were subjected to multimodal CARS microscopy to visualize accumulation of lipid droplet (white color) and intracellular curcumin (green color). Image dimensions: 125×125 μm. Data are presented as means±S.E.M., $n=9$ ($^{**}P<.01$; $^{***}P<.001$), and the experiment was repeated at least twice with similar results.

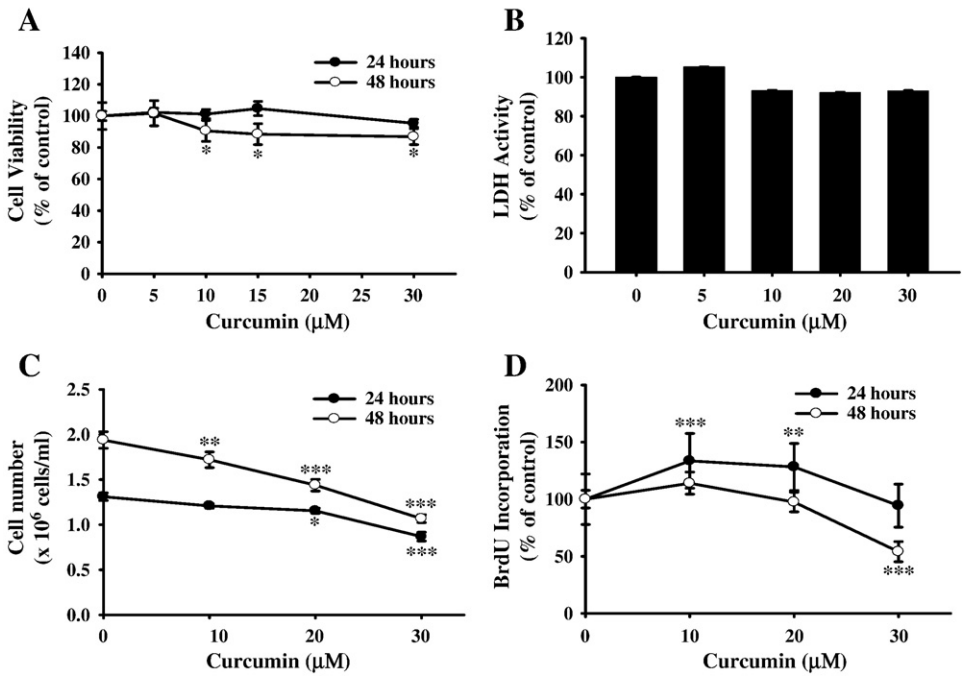

Fig. 3. The effect of curcumin on viability and proliferation of differentiating adipocytes. Two-day postconfluent 3T3-L1 cells were incubated for 24 or 48 h with adipogenic cocktail and various concentrations of curcumin. (A) MTT colorimetric assay for testing cell viability was performed after 24 and 48 h of treatments; (B) LDH activity assay for estimating cytotoxicity was performed after 48 h of treatments; (C) cell number counting using hemocytometer and (D) BrdU incorporation assay were performed after 24 and 48 h of treatments with indicated concentrations of curcumin. Data are presented as means±S.E.M., n=9–36 ($^{*}P<.05$; $^{**}P<.01$; $^{***}P<.001$).

could be due to curcumin-inhibited viability of differentiating 3T3-L1 cells in the early stage of adipocyte differentiation. To test the role of curcumin in the early stage of adipocyte differentiation, we first examined the impact of curcumin on the viability and cytotoxicity of 3T3-L1 cells in the early stage of adipocyte differentiation. Differentiating 3T3-L1 cells treated with various concentrations of curcumin (0–30 μM) from Days 0 to 1 and Days 0 to 2 resulted in no effect and approximately 10% decrease in cell viability, respectively (Fig. 3A). Consistently, we also observed that curcumin treatment from Days 0 to 2 exerts no significant cytotoxic effect on differentiating 3T3-L1 cells treated as assessed by the LDH assay (Fig. 3B). As 3T3-L1 cells incubated with the adipogenic cocktail are known to enter an additional process of cell proliferation in the early stage of adipocyte differentiation, we next tested the effect of curcumin on the proliferation of differentiating 3T3-L1 cells by performing cell number counting assay. Two-day postconfluent 3T3-L1 cells with initial cell number of 8×10^5 cells/ml treated with the adipogenic cocktail in the presence or absence of curcumin (0–30 μM) during Days 0–2 were subjected to the cell number counting assay. The cell number of control cells was reached to 1.3×10^6 and 2×10^6 cells/ml at Day 1 and Day 2, respectively (Fig. 3C). Although there was a slight decrease in the measured cell number when 3T3-L1 cells were incubated with adipogenic cocktail and different concentrations of curcumin for 24 h, differentiating cells treated with curcumin for 48 h exhibited a dose-dependent inhibition of cell proliferation with approximately 1.3×10^6 cells/ml at the treatment with 30 μM curcumin (Fig. 3C). Consistent with this finding, 48 h of treatment of differentiating 3T3-L1 cells with various concentrations of curcumin is shown to be associated with reduced level of BrdU incorporation compared with control cells (Fig. 3D). However, a slight increase in BrdU incorporation was observed when differentiating cells were incubated with 10 or 20 μM curcumin for 24 h. These results suggest that curcumin impairs cell proliferation event in the MCE process of differentiating 3T3-L1 cells in the early stage of adipocyte differentiation.

To gain further insight into the mechanism of the inhibitory role of curcumin in the MCE process of differentiating 3T3-L1 cells, differentiating cells treated with various concentrations of curcumin were subjected to flow cytometry assay. 3T3-L1 cells treated with either curcumin (0–30 μM) for 18 and 24 h postinduction of adipocyte differentiation clearly exhibited a delayed entry of cells into S phase and its consequent transition of cell cycle to G2/M phase when compared with control cells (Fig. 4A and B). Since most differentiating cells are known to enter S phase in cell cycle after 18 h of induction of adipocyte differentiation [27], our finding of delayed cell-cycle entry by curcumin treatment raised the question whether this is a consequence of altered expression of cell-cycle regulators, cyclin A and cdk2, which are essential for G1/S transition [28]. To address this question, an immunoblot assay was performed to examine the effect of curcumin on cyclin A and cdk2 protein levels in differentiating cells. It is evident that cyclin A and cdk2 levels were detected in control cells after 18 and 24 h of initiation of adipocyte differentiation. However, 18 and 24 h of curcumin treatment resulted in a dose-dependent inhibition of cyclin A and cdk2 levels in differentiating 3T3-L1 cells (Fig. 4C). Since cdk2 is involved in phosphorylation of C/EBPβ, which is required for its transcriptional activation of PPARγ and C/EBPα during the early stage of adipocyte differentiation [28–30], we next determined the levels of C/EBPβ phosphorylation in curcumin-treated cells. Fig. 4C shows a slight decrease but not significant change in phosphorylation of C/EBPβ at Thr188 in differentiating 3T3-L1 cells treated with curcumin for 18 and 24 h.

Taken together, our results indicate that the anti-adipogenic function of curcumin is largely mediated through inhibition of proliferation and cell division events in the MCE process during the early stage of adipocyte differentiation with little effect on cell viability and cytotoxicity.

3.4. Curcumin inhibits the expression of C/EBPα and PPARγ mRNAs in the early stage of adipocyte differentiation

Since MCE is found to be required for subsequent activation of transcriptional cascade for terminal adipocyte differentiation, we next

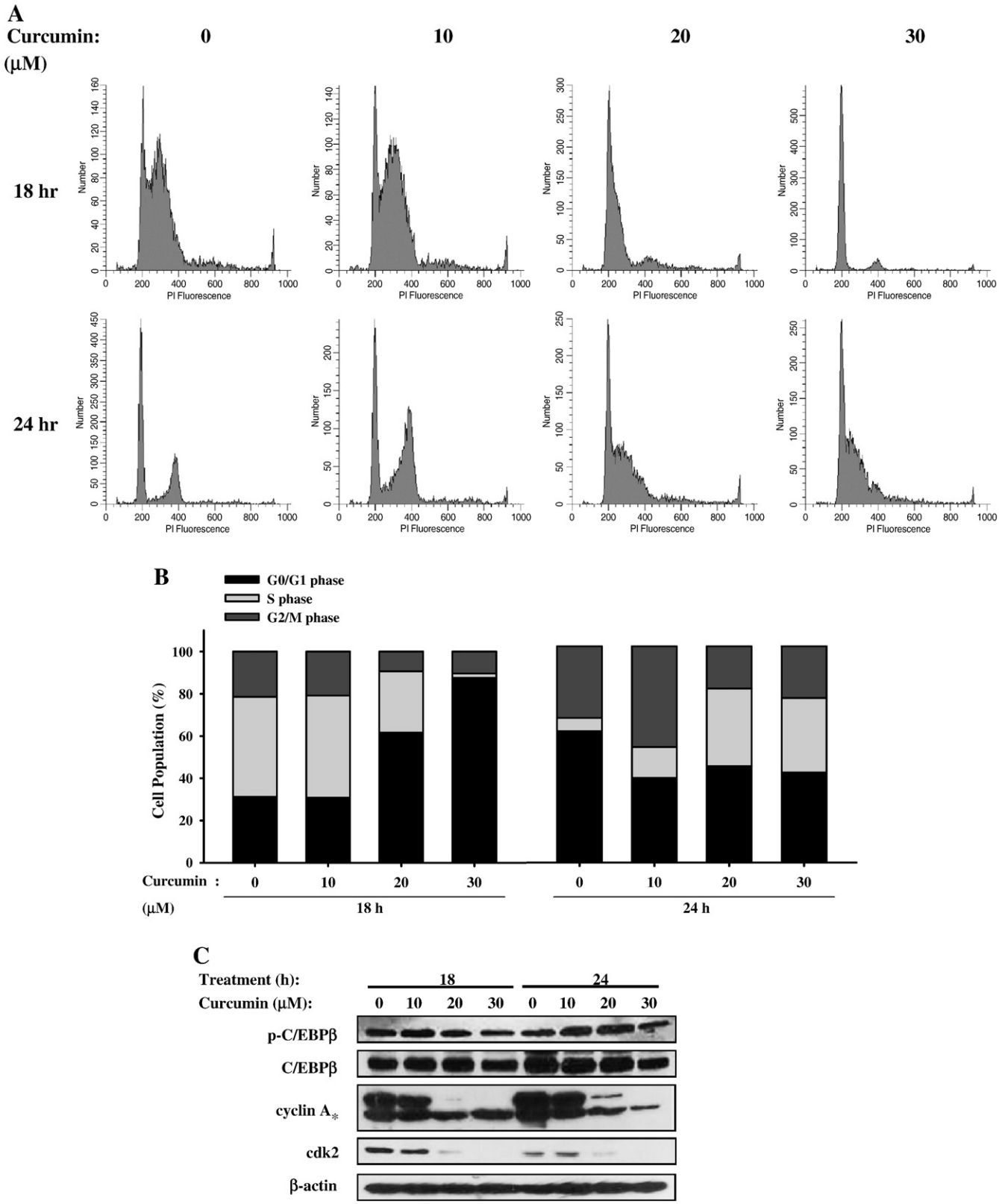

Fig. 4. Cell-cycle analysis of curcumin-treated 3T3-L1 cells during the MCE process of adipocyte differentiation. Two-day postconfluent 3T3-L1 cells were incubated with the adipogenic cocktail in the presence or absence of various concentrations of curcumin. After 18 and 24 h of treatments, differentiating cells were stained with propidium iodine (PI). These cells were then subjected to flow cytometric cell-cycle analysis (A) and quantitative analysis of cells in different phases in cell cycle (B). Data were analyzed using Quanta software. (C) The protein levels of phosphorylated C/EBPβ (p-C/EBPβ), C/EBPβ, cyclin A and cdk2 in these cells were detected by immunoblot assay using their specific antibodies. β-Actin was used as a loading control. An asterisk (*) indicates nonspecific bands. The experiment was repeated at least twice with similar results.

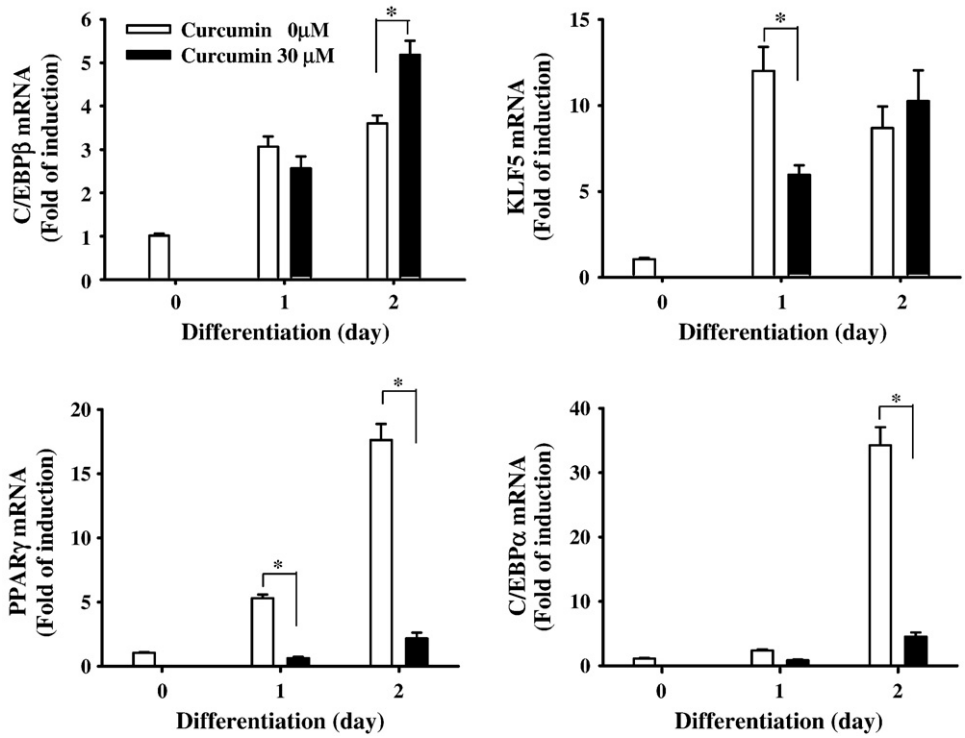

Fig. 5. Curcumin suppresses adipogenic transcription factor expressions. Two-day postconfluent 3T3-L1 cells were incubated with the adipogenic cocktail in the presence or absence of 30 μM of curcumin. Cells harvested at Days 0, 1 and 2 were subjected to quantitative gene expression analysis of C/EBPβ, KLF5, PPARγ and C/EBPα by real-time RT-PCR. The signals were normalized to β-actin internal control, and the results were expressed as relative fold of induction. Data are presented as means±S.E.M., $n=12$ ($^{*}P<.05$).

examined the effect of curcumin on mRNA levels of the early adipogenic transcription factors, including C/EBPβ, KLF5, PPARγ and C/EBPα, in 3T3-L1 cells treated with the adipogenic cocktail in the presence or absence of curcumin for 2 days by real-time RT-PCR. Consistent with the result shown in Fig. 4C, curcumin treatment had no significant effect on the expression of C/EBPβ mRNA at Day 1 in the early stage of adipocyte differentiation, but an increased level of C/EBPβ mRNA was observed at Day 2 (Fig. 5). We also observed a reduced level of KLF5 mRNA in differentiating 3T3-L1 cells treated with curcumin by 75% at only Day 1. Furthermore, mRNA levels of C/EBPα and PPARγ in curcumin-treated cells at Days 1 and 2 were dramatically decreased by approximately 80–90% compared with those in control cells (Fig. 5). These results indicate that curcumin inhibits the expression of adipogenic transcription factors KLF5, PPARγ and C/EBPα in the early stage of adipocyte differentiation, at least in part, due to a consequence of curcumin-inhibited MCE process, which in turn further inhibits terminal adipocyte differentiation.

3.5. Rosiglitazone, a PPARγ ligand, partially rescues adipocyte differentiation of curcumin-treated cells

PPARγ is found to be required for coordinately initiating terminal adipocyte differentiation even if MCE process is altered [3,4,31]. Moreover, overexpression of PPARγ alone is shown to be enough to induce adipocyte differentiation [32]. Since our finding indicates that curcumin-inhibited adipocyte differentiation is associated with a marked decrease in PPARγ mRNA expression, we investigated whether rosiglitazone, a synthetic PPARγ ligand, alone could reverse the inhibitory effect of curcumin on adipocyte differentiation. To address this, adipocyte differentiation of the postconfluent 3T3-L1 preadipocytes was initiated by medium containing the adipogenic cocktail for 2 days in the presence or absence of curcumin and/or 1 μM rosiglitazone. Cells were further differentiated until Day 6 in the absence of curcumin and/or rosiglitazone. The ability of rosiglitazone in rescuing curcumin-inhibited adipocyte differentiation was assessed by intracellular lipid droplet analysis and quantitative analysis of mRNA levels of adipogenic transcription factors. As expected, administration of rosiglitazone to the control 3T3-L1 cells resulted in an increase in adipocyte differentiation (Fig. 6). Moreover, administration of rosiglitazone to 3T3-L1 cells treated with 20 μM curcumin completely reversed curcumin-inhibited adipocyte differentiation (Fig. 6A). Consistent with this, rosiglitazone treatment resulted in elevated mRNA levels of C/EBPβ, PPARγ and C/EBPα in cells treated with 20 μM curcumin to the levels similar to or greater than those in control cells differentiated with rosiglitazone (Fig. 6B–D). Although rosiglitazone treatment elevated levels of PPARγ and C/EBPα mRNA in adipocytes treated with 30 μM curcumin to the levels similar to or greater than those in control cells differentiated with rosiglitazone (Fig. 6B–D), rosiglitazone treatment failed to rescue adipocyte differentiation in these cells, as judged by quantitative analysis of ORO staining (Fig. 6A). These results indicate that rosiglitazone alone appears to partly overcome the inhibitory effect of curcumin on adipocyte differentiation.

4. Discussion

In the present study, we have identified curcumin as an effective anti-adipogenic compound in cultured mouse and human preadipocytes (Fig. 1). We further elucidated that the presence of curcumin during the first 2 days of adipocyte differentiation plays a critical role in inhibiting adipocyte differentiation (Fig. 2). This result suggests that the anti-adipogenic function of curcumin is likely through modulating cellular events that occurred in the early stage of adipocyte differentiation, but to a lesser extent in the postmitotic intermediate and the terminal stages of adipocyte differentiation. As determined by multimodal CARS microscope, the anti-adipogenic function of curcumin is correlated with the presence of curcumin in

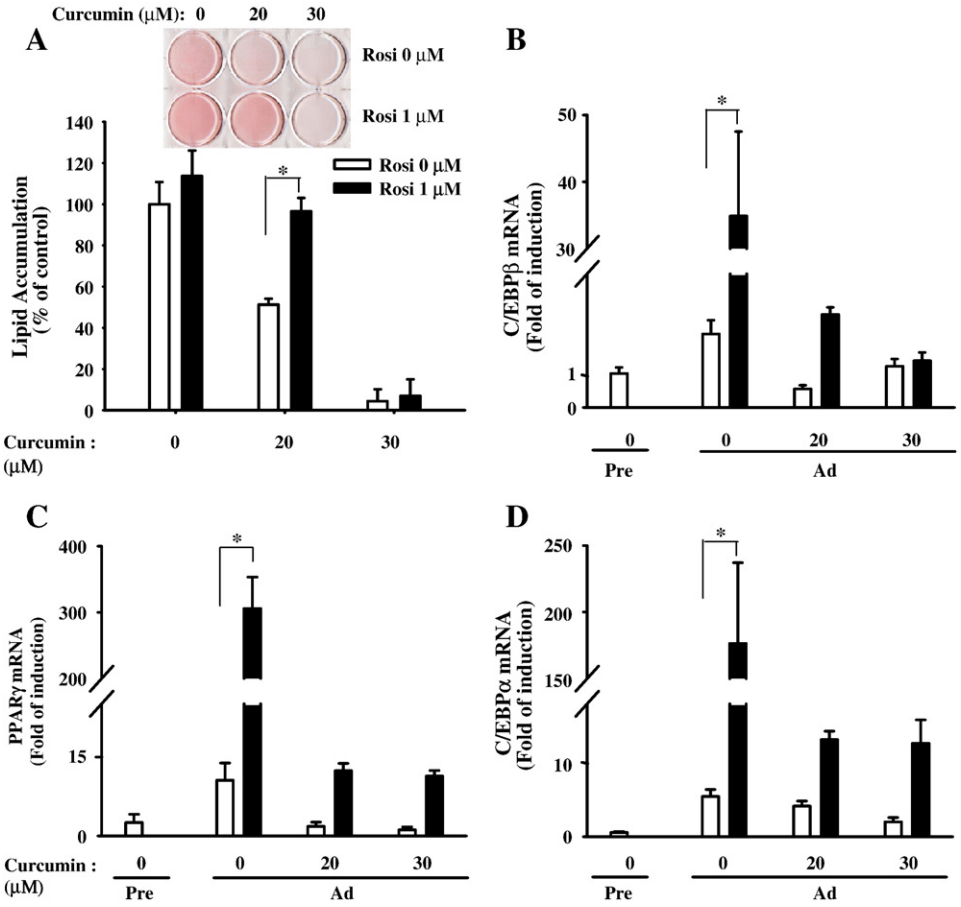

Fig. 6. Rosiglitazone partially rescues curcumin-inhibited adipocyte differentiation. Two-day postconfluent 3T3-L1 cells were incubated with the adipogenic cocktail in the absence or presence of 1 μM rosiglitazone and/or curcumin (0–30 μM) for 2 days in the early stage of adipocyte differentiation, followed by additional 4 days of adipocyte differentiation in the absence of rosiglitazone and/or curcumin. (A) Six days after differentiation, cells were stained with ORO and the amount of ORO-stained intracellular lipids was quantified. (B–D) Total RNA isolated from differentiated adipocytes (Ad) at Day 6 or from undifferentiated preadipocytes (Pre) was subjected to quantitative gene expression analysis of C/EBPβ, PPARγ and C/EBPα by real-time RT-PCR. The signals were normalized to β-actin internal control, and the results were expressed as relative fold of induction. Data are presented as means±S.E.M., n=4–12 ($^{*}P$<.05).

differentiated adipocytes (Fig. 2D). Moreover, a 3-D CARS imaging analysis of these cells further revealed that the fluorescent green signal of curcumin was from intracellular compartments, not from the surface membrane of adipocytes (data not shown).

Curcumin has been shown to inhibit cell viability and to promote apoptosis of colon, skin and breast tumors both *in vitro* and *in vivo* [25,26,33,34]. Thus, we first tested whether curcumin-inhibited adipocyte differentiation is due to its known inhibitory role in cell viability. In consistency with its known cytotoxic effect, we also observed that curcumin is able to inhibit viability of proliferating preadipocytes (data not shown). However, we observed that curcumin (0–30 μM) has little on viability and cytotoxicity in differentiating 3T3-L1 cells (Fig. 3A and B). We further demonstrated that curcumin inhibits MCE process in the early stage of adipocyte differentiation as evidenced by a delayed cell-cycle progression, a significantly decreased number of cells in the S phase and G2/M phase at 18 h postinduction of differentiation, and suppressed cyclin A and cdk2 levels in curcumin-treated cells during the early stage of adipocyte differentiation (Fig. 3C and D and Fig. 4). It should be noted that differentiating 3T3-L1 cells treated with different concentrations of curcumin (0–20 μM) resulted in an increase in BrdU incorporation (Fig. 3D). Although the mechanism by which low concentration of curcumin induces BrdU incorporation in differentiating adipocytes is unknown, similar results had been reported in non-adipocytes such as neural progenitor cells and colon epithelial cells [35,36]. Since BrdU incorporation is known to monitor new synthesis of DNA and the S phase of the cell cycle, we propose that the increased BrdU incorporation in differentiating adipocytes treated with curcumin at 10 and 20 μM is likely due to having more cells arrested in the S phase and/or G2 phase in the cell cycle.

Since postconfluent DNA replication, cell division and cell proliferation during MCE are known to be required for initiation of transcriptional cascade of adipocyte differentiation, our data suggest that the anti-adipogenic function of curcumin is largely through its inhibitory role in MCE process. Of note, recent studies suggest that C/EBPβ is the likely link between MCE and transcriptional activation of KLF5, PPARγ and C/EBPα, in the early stage of adipocyte differentiation [37]. This is known to be mediated through localization of C/EBPβ to centromeres of preadipocytes 12–16 h postinduction of adipocyte differentiation (i.e., S phase) and its subsequent phosphorylation by MCE-associated mitogen-activated protein kinase and glycogen synthase kinase 3β [28,38]. However, both protein (Fig. 4C) and mRNA (Fig. 5A) levels of C/EBPβ were not affected by curcumin within 24 h of initiation of adipocyte differentiation. Interestingly, we observed a dramatic decrease in mRNA levels of KLF5 at Day 1 and of PPARγ and C/EBPα at Days 1 and 2 in the early stage of adipocyte differentiation (Fig. 5). Since KLF5 is known to be required for adipocyte differentiation with its role in regulation of PPARγ and C/EBPα through a coordinate action with C/EBPβ/δ in the early stage of adipocyte differentiation [39], our finding of curcumin-suppressed mRNA

expression of PPARγ and C/EBPα in the early stage of adipocyte differentiation is likely to be a consequence of the function of KLF5 and/or C/EBPβ function (e.g., localization to centromeres and DNA binding ability) in the early stage of adipocyte differentiation.

Rosiglitazone is a very potent adipogenic inducer [3,4]. Our study shows that supplementation with rosiglitazone during the early stage of adipocyte differentiation partially rescued curcumin-inhibited adipocyte differentiation as it fully reverses the differentiation of 3T3-L1 cells when they are treated with curcumin up to 20 μM, but it shows no effect on 30 μM curcumin-treated cells (Fig. 6). The different responsiveness of adipocytes to various concentrations of curcumin was also seen in Figs. 3 and 4 where treatment of 20 μM curcumin showed less significant inhibitory effects on proliferation and cell-cycle entry of differentiating adipocytes than that of 30 μM curcumin. As MCE process is known to precede the expression of the adipogenic transcription factor PPARγ, we believe that a forced activation of PPARγ is not enough to overcome altered MCE and inhibited adipocyte differentiation by treatment with high concentration of curcumin.

Recently, the therapeutic and preventive function of curcumin in obesity has been tested in animal models of obesity [14,15]. Weisberg et al. suggested that curcumin is an effective therapeutic agent for improving glycemic response and inflammation in obese and diabetic mice [14]. Ejaz et al. [15] demonstrated a potential preventive function of curcumin in an animal model of diet-induced obesity with suppressed adipocyte differentiation and reduced mRNA levels of PPARγ and C/EBPα in curcumin-treated adipocytes, which is in agreement with our finding (Fig. 5). Curcumin-inhibited adipocyte differentiation was also reported by Lee et al. [44], but the detailed molecular basis underlying curcumin-inhibited adipocyte differentiation was not fully understood. During the submission of this manuscript, Ahn et al. [40] also reported an inhibitory role of curcumin in adipocyte differentiation largely through prevention of adipocyte differentiation-associated inhibition of Wnt signaling. Since Wnt signaling has been shown to be involved in regulation of the early stage of adipocyte differentiation [41] and is likely to be the downstream target of various cell-cycle regulators such as cdk2 and cyclin A [42,43], it will be of interest to study the role of curcumin in the connection between Wnt signaling and MCE during the early stage of adipocyte differentiation.

Our study proposes that a complete inhibition of adipocyte differentiation by curcumin requires concentrations up to 30 μM *in vitro*, and this is in consistency with the concentration ranges used in recent *in vitro* studies [15,40,44]. Our IC_{50} of curcumin in adipocyte differentiation is approximately 16.1 and 11.1 μM in murine 3T3-L1 cells and human primary preadipocytes, respectively. Given that oral administration of curcumin to animals has shown to contribute to blood plasma curcumin concentration in the range from nanomolar to near 13 μM depending on study designs [45–47], it is possible that the IC_{50} of curcumin estimated in our study may not be fully achieved in the blood of animals fed diet containing curcumin. However, it has been demonstrated that a chronic supplementation of high-fat diet in mice with a practical dose of curcumin at 0.05% in diet is able to reduce body weight gain and adiposity with a potential inhibitory role in differentiation and lipid metabolism of adipocytes [15]. This suggests that adipocytes in the physiological condition could be more sensitive to chronic exposure of low concentration of circulating curcumin to exert a beneficial function than those used in *in vitro* studies. Although curcumin could exert an anti-obesity function *in vivo* through modulating various cellular pathways, it is reasonable to propose that our finding of an inhibitory role of curcumin in MCE process during adipocyte differentiation is likely to be a mechanism underlying the lowering adipogenic capacity of adipose tissue, thereby preventing adiposity at low physiological dose of curcumin.

In summary, our cellular/biochemical study combined with molecular imaging analysis clearly demonstrates that curcumin-inhibited adipocyte differentiation is through the inhibition of MCE process, thereby lowering the expression of early adipogenic transcription factors KLF5, PPARγ and C/EBPα mRNAs in adipocytes during the early stage of cell differentiation. Our results also indicate that activation of PPARγ function is not sufficient enough to reverse the inhibitory function of curcumin in adipocyte differentiation. Taken together, our study provides new insights into the molecular basis underlying the anti-adipogenic property of curcumin.

Acknowledgments

We are grateful to Allison Martorano and Julie Wiacek in the Kim laboratory for critical comments on the manuscript.

References

[1] Kopelman PG. Obesity as a medical problem. Nature 2000;404:635–43.
[2] Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature 2008;453:783–7.
[3] Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. Physiol Rev 1998;78:783–809.
[4] Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 2006;7:885–96.
[5] Ammon HP, Wahl MA. Pharmacology of *Curcuma longa*. Planta Med 1991;57:1–7.
[6] Corson TW, Crews CM. Molecular understanding and modern application of traditional medicines: triumphs and trials. Cell 2007;130:769–74.
[7] Singh S. From exotic spice to modern drug? Cell 2007;130:765–8.
[8] Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. Biochem Pharmacol 2008;75:787–809.
[9] Feng B, Chen S, Chiu J, George B, Chakrabarti S. Regulation of cardiomyocyte hypertrophy in diabetes at the transcriptional level. Am J Physiol Endocrinol Metab 2008;294:E1119–26.
[10] Li HL, Liu C, de Couto G, Ouzounian M, Sun M, Wang AB, et al. Curcumin prevents and reverses murine cardiac hypertrophy. J Clin Invest 2008;118:879–93.
[11] Morimoto T, Sunagawa Y, Kawamura T, Takaya T, Wada H, Nagasawa A, et al. The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. J Clin Invest 2008;118:868–78.
[12] Wongcharoen W, Phrommintikul A. The protective role of curcumin in cardiovascular diseases. Int J Cardiol 2009;133:145–51.
[13] Seo KI, Choi MS, Jung UJ, Kim HJ, Yeo J, Jeon SM, et al. Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. Mol Nutr Food Res 2008;52:995–1004.
[14] Weisberg SP, Leibel R, Tortoriello DV. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology 2008;149:3549–58.
[15] Ejaz A, Wu D, Kwan P, Meydani M. Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. J Nutr 2009;139:919–25.
[16] Kim KH, Lee K, Moon YS, Sul HS. A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J Biol Chem 2001;276:11252–6.
[17] Zhu J, Lee B, Buhman KK, Cheng JX. A dynamic, cytoplasmic triacylglycerol pool in enterocytes revealed by ex vivo and in vivo coherent anti-Stokes Raman scattering imaging. J Lipid Res 2009;50:1080–9.
[18] Le TT, Cheng JX. Single-cell profiling reveals the origin of phenotypic variability in adipogenesis. PLoS ONE 2009;4:e5189.
[19] Wang HW, Le TT, Cheng JX. Label-free imaging of arterial cells and extracellular matrix using a multimodal CARS microscope. Opt Commun 2008;281:1813–22.
[20] Fu Y, Huff TB, Wang HW, Wang H, Cheng JX. Ex vivo and in vivo imaging of myelin fibers in mouse brain by coherent anti-Stokes Raman scattering microscopy. Opt Express 2008;16:19396–409.
[21] Nan X, Cheng JX, Xie XS. Vibrational imaging of lipid droplets in live fibroblast cells with coherent anti-Stokes Raman scattering microscopy. J Lipid Res 2003;44:2202–8.
[22] Evans CL, Xie XS. Coherent anti-stokes Raman scattering microscopy: chemical imaging for biology and medicine. Annual Review of Analytical Chemistry 2008;1:883–909.
[23] Wang HW, Fu Y, Huff TB, Le TT, Wang H, Cheng JX. Chasing lipids in health and diseases by coherent anti-Strokes Raman scattering microscopy. Vibrational Spectroscopy 2009;50:160–7.
[24] Mendonca LM, Dos Santos GC, Antonucci GA, Dos Santos AC, Bianchi Mde L, Antunes LM. Evaluation of the cytotoxicity and genotoxicity of curcumin in PC12 cells. Mutat Res 2009;675:29–34.
[25] Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 2003;23:363–98.
[26] Menon LG, Kuttan R, Kuttan G. Inhibition of lung metastasis in mice induced by B16F10 melanoma cells by polyphenolic compounds. Cancer Lett 1995;95:221–5.

[27] Tang QQ, Lane MD. Activation and centromeric localization of CCAAT/enhancer-binding proteins during the mitotic clonal expansion of adipocyte differentiation. Genes Dev 1999;13:2231–41.
[28] Li X, Kim JW, Gronborg M, Urlaub H, Lane MD, Tang QQ. Role of cdk2 in the sequential phosphorylation/activation of C/EBPbeta during adipocyte differentiation. Proc Natl Acad Sci U S A 2007;104:11597–602.
[29] Tang QQ, Gronborg M, Huang H, Kim JW, Otto TC, Pandey A, et al. Sequential phosphorylation of CCAAT enhancer-binding protein beta by MAPK and glycogen synthase kinase 3beta is required for adipogenesis. Proc Natl Acad Sci U S A 2005;102:9766–71.
[30] Tang QQ, Otto TC, Lane MD. CCAAT/enhancer-binding protein beta is required for mitotic clonal expansion during adipogenesis. Proc Natl Acad Sci U S A 2003;100: 850–5.
[31] Cho YC, Jefcoate CR. PPARgamma1 synthesis and adipogenesis in C3H10T1/2 cells depends on S-phase progression, but does not require mitotic clonal expansion. J Cell Biochem 2004;91:336–53.
[32] Iwata M, Haruta T, Usui I, Takata Y, Takano A, Uno T, et al. Pioglitazone ameliorates tumor necrosis factor-alpha-induced insulin resistance by a mechanism independent of adipogenic activity of peroxisome proliferator-activated receptor-gamma. Diabetes 2001;50:1083–92.
[33] Johnson JJ, Mukhtar H. Curcumin for chemoprevention of colon cancer. Cancer Lett 2007;255:170–81.
[34] Shishodia S, Amin HM, Lai R, Aggarwal BB. Curcumin (diferuloylmethane) inhibits constitutive NF-kappaB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma. Biochem Pharmacol 2005;70: 700–13.
[35] Kim SJ, Hellerstein MK. Pharmacological doses of dietary curcumin increase colon epithelial cell proliferation in vivo in rats. Phytother Res 2007;21:995–8.
[36] Kim SJ, Son TG, Park HR, Park M, Kim MS, Kim HS, et al. Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus. J Biol Chem 2008;283:14497–505.
[37] Tang QQ, Otto TC, Lane MD. Mitotic clonal expansion: a synchronous process required for adipogenesis. Proc Natl Acad Sci U S A 2003;100:44–9.
[38] Park BH, Qiang L, Farmer SR. Phosphorylation of C/EBPbeta at a consensus extracellular signal-regulated kinase/glycogen synthase kinase 3 site is required for the induction of adiponectin gene expression during the differentiation of mouse fibroblasts into adipocytes. Mol Cell Biol 2004;24:8671–80.
[39] Oishi Y, Manabe I, Tobe K, Tsushima K, Shindo T, Fujiu K, et al. Kruppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. Cell Metab 2005;1:27–39.
[40] Ahn J, Lee H, Kim S, Ha T. Curcumin-induced suppression of adipogenic differentiation is accompanied by activation of Wnt/beta-catenin signaling. Am J Physiol Cell Physiol 2010;298:C1510–1516.
[41] Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, et al. Inhibition of adipogenesis by Wnt signaling. Science 2000;289:950–3.
[42] Kim SI, Park CS, Lee MS, Kwon MS, Jho EH, Song WK. Cyclin-dependent kinase 2 regulates the interaction of Axin with beta-catenin. Biochem Biophys Res Commun 2004;317:478–83.
[43] Park CS, Kim SI, Lee MS, Youn CY, Kim DJ, Jho EH, et al. Modulation of beta-catenin phosphorylation/degradation by cyclin-dependent kinase 2. J Biol Chem 2004;279:19592–9.
[44] Lee YK, Lee WS, Hwang JT, Kwon DY, Surh YJ, Park OJ. Curcumin exerts antidifferentiation effect through AMPKalpha-PPAR-gamma in 3T3-L1 adipocytes and antiproliferatory effect through AMPKalpha-COX-2 in cancer cells. J Agric Food Chem 2009;57:305–10.
[45] Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med 1998;64:353–6.
[46] Ravindranath V, Chandrasekhara N. Absorption and tissue distribution of curcumin in rats. Toxicology 1980;16:259–65.
[47] Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. Drug Metab Dispos 1999;27:486–94.