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Am J Physiol Cell Physiol 298:C1510-C1516, 2010. First published 31 March 2010;
doi:10.1152/ajpcell.00369.2009

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Submitted 14 August 2009; accepted in final form 28 March 2010

Ahn J, Lee H, Kim S, Ha T. Curcumin-induced suppression of adipogenic differentiation is accompanied by activation of Wnt/β-catenin signaling. *Am J Physiol Cell Physiol* 298: C1510–C1516, 2010. First published March 31, 2010; doi:10.1152/ajpcell.00369.2009.—Curcumin, a polyphenol found in the rhizomes of *Curcuma longa*, improves obesity-associated inflammation and diabetes in obese mice. Curcumin also suppresses adipocyte differentiation, although the underlying mechanism remains unclear. Here, we used 3T3-L1 cells to investigate the details of the mechanism underlying the anti-adipogenic effects of curcumin. Curcumin inhibited mitogen-activated protein kinase (MAPK) (ERK, JNK, and p38) phosphorylation that was associated with differentiation of 3T3-L1 cells into adipocytes. During differentiation, curcumin also restored nuclear translocation of the integral Wnt signaling component β-catenin in a dose-dependent manner. In parallel, curcumin reduced differentiation-stimulated expression of CK1α, GSK-3β, and Axin, components of the destruction complex targeting β-catenin. Accordingly, quantitative PCR analysis revealed that curcumin inhibited the mRNA expression of AP2 (mature adipocyte marker) and increased the mRNA expression of Wnt10b, Fz2 (Wnt direct receptor), and LRP5 (Wnt coreceptor). Curcumin also increased mRNA levels of c-Myc and cyclin D1, well-known Wnt targets. These results suggest that the Wnt signaling pathway participates in curcumin-induced suppression of adipogenesis in 3T3-L1 cells.

adipogenesis; mitogen-activated protein kinase; 3T3-L1 cells

OBESITY is an energy balance disorder in which nutrient intake chronically exceeds energy expenditure, resulting in excessive white adipose tissue accumulation. Obesity is frequently associated with insulin resistance, which is in turn linked to the development of type 2 diabetes, hypertension, hyperlipidemia, and atherosclerosis-the so-called metabolic syndrome (22). Obesity is also associated with a substantially decreased health-related quality of life and increased medical expenditures (1, 6, 13). Adipocytes are key inducers of insulin resistance by exerting lipotoxicity and modulating adipokine secretion. Thus understanding the molecular and cellular events regulating adipogenesis is crucial for designing rational therapies to prevent and treat obesity and the related metabolic syndrome.

The Wnt/β-catenin signaling pathway affects multiple cellular functions by regulating β-catenin levels and subcellular localization of β-catenin (48). Wnt proteins are lipid-modified proteins that, when secreted, activate cell surface receptor-mediated signal transduction pathways to regulate a variety of cellular activities, including cell growth and cell fate determination (3). In the absence of Wnt-induced signal transduction, glycogen synthase kinase 3β (GSK3β), Axin, adenomatous polyposis coli, and casein kinase 1α (CK1α) form a β-catenin destruction complex. β-Catenin is the molecular node of the canonical Wnt signaling pathway (48). CK1α phosphorylates β-catenin at ^{45}Ser, whereas GSK3β phosphorylates β-catenin at ^{33}Ser/^{37}Ser/^{41}Thr (29). Active GSK-3β also phosphorylates Axin, which in turn recruits β-catenin into the Axin/GSK-3β complex (34). Protein phosphatase 2A (PP2A) then facilitates the release of phosphorylated β-catenin from the destruction complex, allowing for its ubiquitination and subsequent degradation (49). Wnt/β-catenin signaling occurs when Wnt proteins bind to a member of the seven transmembrane domain-containing Frizzled (Fz) receptor family. Fz then heterodimerizes with a member of the low-density lipoprotein-related protein (LRP) family and activates Dishevelled. Dishevelled antagonizes the ability of constitutively active GSK3β to phosphorylate β-catenin and inhibits its association with Axin, preventing formation of the destruction complex. Thus, once β-catenin is hypophosphorylated due to Wnt signaling, it is stabilized and translocates to the nucleus where it binds the T-cell factor (TCF)/lymphoid-enhancer factor family of transcription factors to regulate the expression of Wnt target genes (3, 31). MacDougald et al. (2, 40) have reported that Wnt signaling functions as an adipogenesis switch that represses adipogenesis when activated and initiates adipogenesis when turned off. They demonstrated that Wnt signaling, possibly mediated by Wnt10b, maintains preadipocytes in an undifferentiated state via inhibition of the key adipogenic transcription factors, CCAAT/enhancer binding protein-α (C/EBPα), and peroxisome proliferator-activated receptor-γ (PPARγ).

Curcumin is a low-molecular-weight polyphenol derived from the herbal remedy and dietary spice turmeric. Turmeric, dried ground rhizomes of the perennial herb *Curcuma longa*, is a popular dietary spice in Asia and is used in curry. Curcumin is generally regarded as the most active constituent of turmeric and comprises 2–8% of most turmeric preparations. This compound exhibits anti-inflammatory activities (12) and decreases blood glucose levels in type 2 diabetic KK-A(y) mice (34). Curcumin modulates the inflammatory responses of adipose tissue macrophages and adipocytes (50). Several in vitro studies have demonstrated that curcumin also inhibits adipogenesis in 3T3-L1 cells (8, 27), and Ejaz et al. (8) showed that 5′-AMP-activated protein kinase is involved in this effect. However, the role of the Wnt signaling pathway in mediating the suppressive effect of curcumin on adipogenic differentiation has not yet been investigated. In this study, we investigated the role of Wnt/β-catenin signaling in the anti-adipogenic activity of curcumin in 3T3-L1 cells.

MATERIALS AND METHODS

Materials. 3T3-L1 fibroblast cells were purchased from the American Type Culture Collection. IBMX, dexamethasone, insulin, and Oil red O were purchased from Sigma-Aldrich Chemical. DMEM, FBS,

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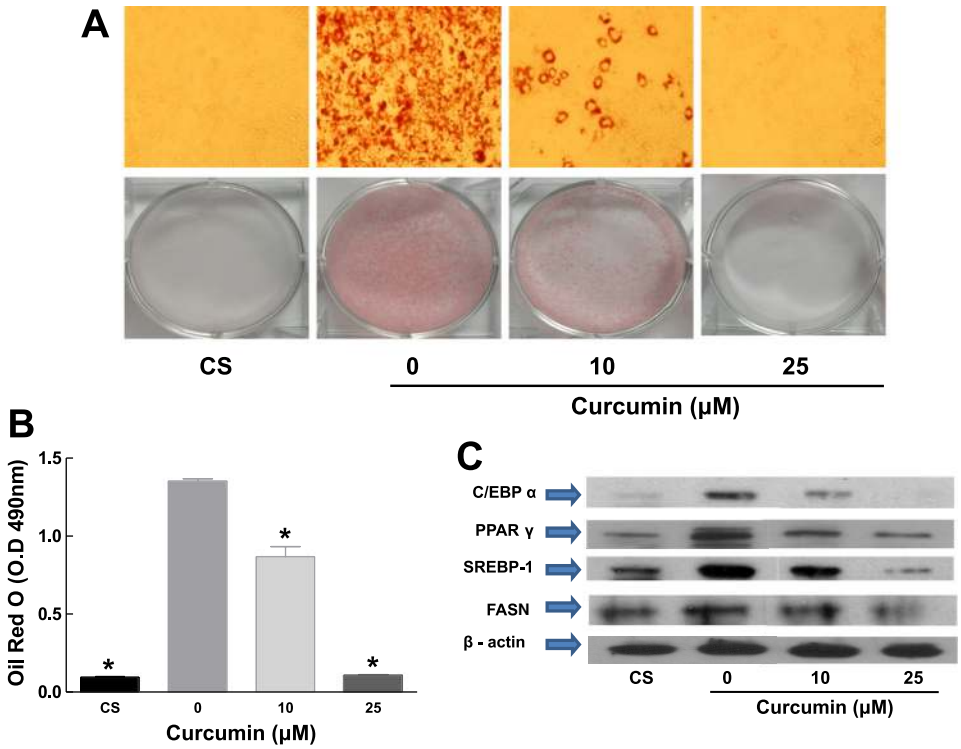

Fig. 1. Curcumin inhibits the adipogenic differentiation of 3T3-L1 preadipocytes. *A*: Oil red O staining of 3T3-L1 cells that were induced to differentiate for 10 days and treated with 10 or 25 μM curcumin at *day 3* for 48 h. Nondifferentiated cells were incubated in DMEM with 10% calf serum (CS). Representative photomicrographs (×200) are shown for each treatment group. *B*: quantitative analysis of adipocyte differentiation, as assessed by spectrophotometric measurement of Oil Red O-stained adipocytes. $*P < 0.01$ vs. control. *C*: Western blot analysis of adipogenic transcription factors and their targets in 3T3-L1 cells. β-Actin served as an internal control.

bovine calf serum, and penicillin-streptomycin were purchased from GIBCO (Invitrogen). Anti-PPARγ, anti-C/EBPα, anti-sterol regulatory element binding protein (SREBP-1), anti-fatty acid synthase (FASN), and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG were obtained from Santa Cruz Biotechnology. Anti-ERK1/2, anti-phosphorylated ERK1/2, anti-JNK, anti-phosphorylated JNK, anti-p38, anti-phosphorylated p38, anti-β-catenin, anti-Axin, anti-CK1α, anti-GSK-3β, anti-c-Myc, and HRP-conjugated anti-mouse IgG were purchased from Cell Signaling Technology. DAPI was purchased from Molecular Probes (Invitrogen). The soluble Wnt inhibitor sFRP-2 was obtained from R&D systems.

Cell culture. Mouse 3T3-L1 fibroblasts were grown at 37°C in a humidified atmosphere of 5% CO_2 and were maintained in DMEM containing 25 mM glucose, 10% calf serum (CS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Cells were subcultured every three to four days at ~90% confluence. Cells were seeded onto 6- or 12-well plates after one to five passages from the original vial. Cells were plated at a density that allowed them to reach confluence in 3 days. At this point (*day 0*), cells were switched to MDI-differentiation medium (DMEM, 10% FBS, 0.25 μM dexamethasone, 0.25 mM IBMX, and 1 μg/ml insulin) in the presence of 10 or 25 μM curcumin to examine the effect of curcumin on adipogenic differentiation. On *day 3*, the dexamethasone and IBMX were removed, and cells were cultured in insulin-containing cell medium for an additional 2 days, followed by culturing in 10% FBS/DMEM medium for an additional 4 days (*day 8*), at which time >90% of cells were mature adipocytes with accumulated fat droplets. To inactivate extracellular Wnt, 3T3-L1 cells were cultured the presence of 100 nM sFRP-2 for 10 days with or without 25 μM curcumin. Wnt inhibitors were added with medium changes every 2 days. Curcumin was treated only for the first 2 days.

Oil red O staining. The cells were washed twice with ice-cold PBS, fixed with 10% formalin at room temperature (RT) for 1 h, and stained with 0.2% Oil red O in isopropanol for 10 min. Cells were then washed with 60% isopropanol and water. Images were collected using an Olympus (Tokyo, Japan) microscope. Stained oil droplets were dissolved in isopropanol and quantified at 490 nm using a spectrophotometer.

Immunofluorescence staining. Cells were fixed with 4% paraformaldehyde for 20 min, incubated in ice-cold methanol for 5 min at −20°C, and washed with PBS. For β-catenin staining, cells were blocked with normal goat serum and successively incubated overnight with rabbit anti-β-catenin antibody (1:400) at 4°C and Alexa Fluor-conjugated goat anti-rabbit antibody (1:750, Santa Cruz Biotechnology) for 2 h at RT. The cells were then counterstained with DAPI, mounted in prolong fade (Molecular Probes), and examined under a fluorescence microscope (Olympus, Japan) equipped with the appropriate filters. Digital images were recorded with a CCD camera using the ISIS fluorescence image analysis system.

Western blot analysis. Cells were harvested and lysed in RIPA buffer containing 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris·HCl (pH 7.4), 50 mM glycerophosphate, 20 mM

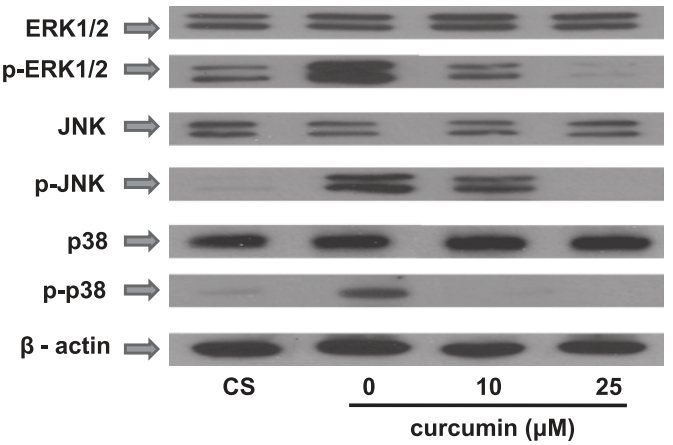

Fig. 2. Effect of curcumin on mitogen-activated protein kaines (MAPK) phosphorylation in differentiated 3T3-L1 cells. Western blot analysis of ERK1/2, phosphorylated (p)-ERK1/2, JNK, p-JNK, p38, p-p38, and β-actin is shown. Cells were treated with 10 or 25 μM curcumin at *day 3* for 48 h. β-Actin served as an internal control.

NaF, 20 mM EGTA, 1 mM DTT, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitors. Nuclear and cytosolic fractions were prepared by using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce, Rockford, IL) according to the manufacturer's instructions. Total cellular protein or subcellular fractions (20 μg) were separated with 12% SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. Equal amounts of protein in each lane were verified by Ponceau S staining. Membranes were incubated with primary antibodies (1:1,000) overnight at 4°C and then with HRP-conjugated secondary antibodies (1:2,000) for 1 h at RT. Immunoreactive proteins were visualized by using an enhanced chemiluminescent reagent (Amersham Pharmacia Biotech).

Real-time PCR. Total RNA was isolated using the RNeasy mini kit (Qiagen) following the manufacturer's instructions, and cDNA synthesis was carried out as described previously (4). For real-time quantitative RT-PCR, 100 ng cDNA was amplified using a LightCycler480 (Roche). β-Actin served as an internal control. The primers were as follows: Wnt10b, sense 5′-ATGCGGATCCACAACAACAG-3′ and anti-sense 5′-TTCCATGGCATTTGCACTTC-3′; Fz2, sense 5′-GTTCTTCTCGCAAGAGGAGAC-3′ and anti-sense 5′-TCGCTGCATGTCCACTAAATAG-3′; LRP5, sense 5′-AAGGGTGCTGTGTACTGGAC-3′ and anti-sense 5′-AGAAGAGAACCTTACGGGACG -3′; Ap2, sense 5′-CCGCAGACGACAGGA-3′ and anti-sense 5′-CTCATGCCCTTTCATAAACT-3′; c-Myc, sense 5′-GCTCGCCCAAATCCTGTA-3′ and anti-sense 5′-AGGACTCGGAGGACAGCA-3′; cyclin D1, sense 5′-CACAACGCACTTTCTTTCCA-3′ and anti-sense 5′-ACCAGCCTCTTCCTCCACTT-3′.

Statistical analysis. Results are presented as means ± SD of three experiments. Statistical analyses were performed using GraphPad Prism 5 software (San Diego, CA). One-way ANOVA was used to compare quantitative data among the groups. The Bonferroni post-hoc test was used to compare group means if the results of the ANOVA were significant ($P < 0.05$).

RESULTS

Curcumin suppresses differentiation-induced upregulation of adipogenic transcription factors and inhibits adipogenesis. Oil red O staining revealed that treating 3T3-L1 fibroblasts with 10 μM curcumin partially inhibited differentiation, whereas 25

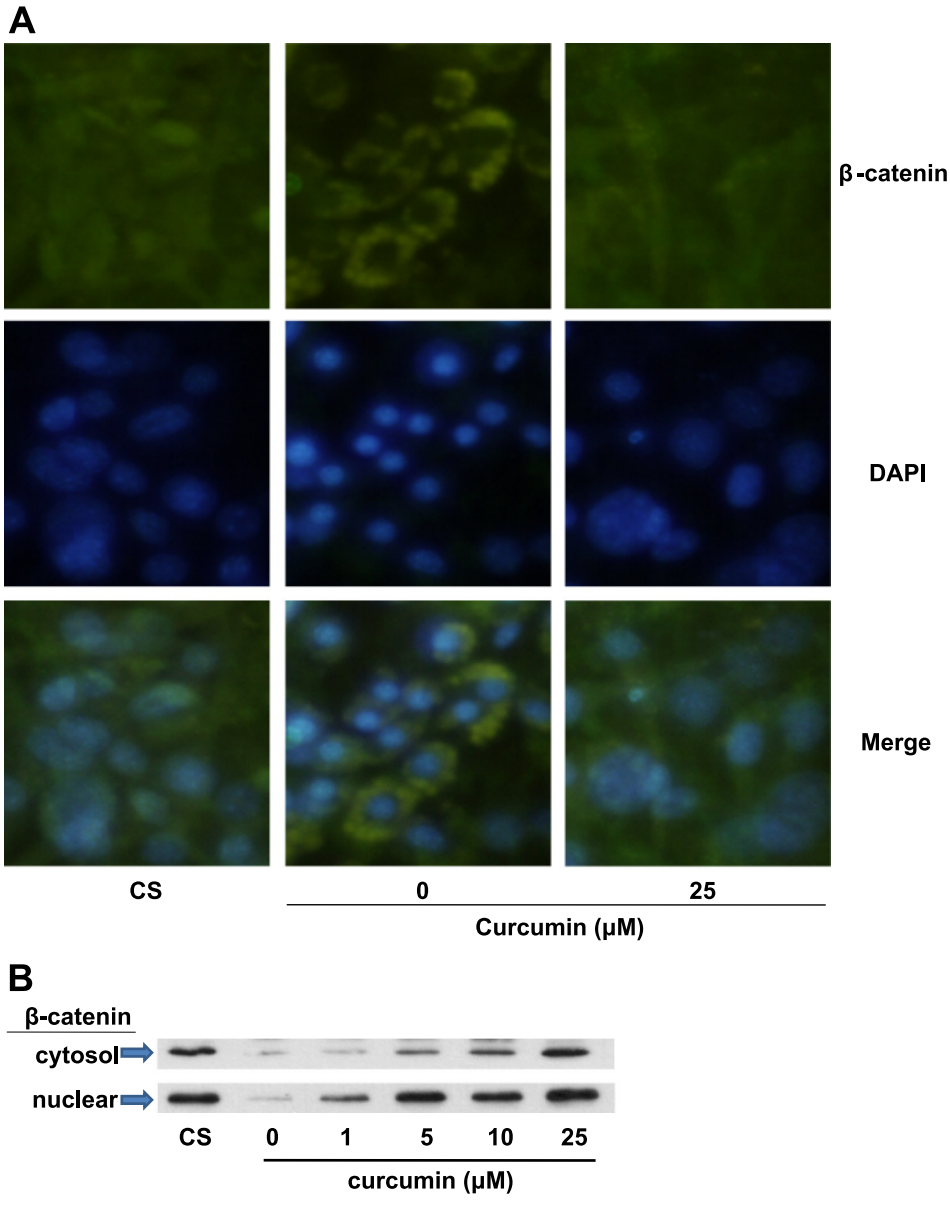

Fig. 3. Curcumin induces the translocation of β-catenin in 3T3-L1 cells. *A*: indirect immunofluorescence staining for β-catenin (green). Nuclei were counterstained with DAPI (blue). Representative photomicrographs (×400) are shown for each treatment group. *B*: Western blot analysis of β-catenin in nuclear and cytosolic fractions is shown. Cells were treated with varying concentrations of curcumin (1–25 μM) for 48 h.

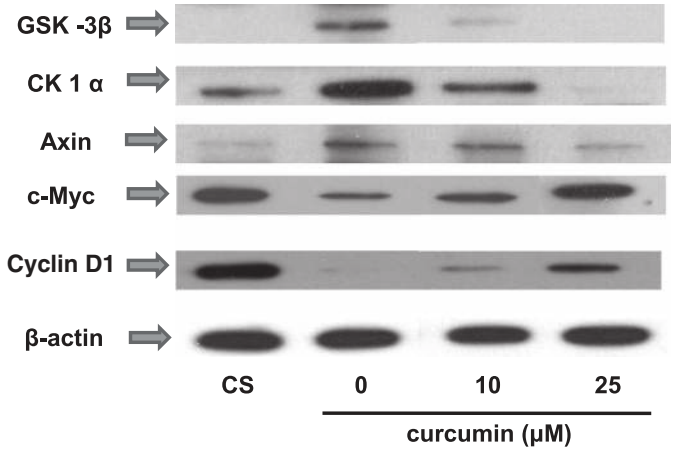

Fig. 4. Effect of curcumin on expression of the β-catenin degradation complex and Wnt targets. Western blot analysis of β-catenin degradation complex components (GSK-3β, CK1α, and Axin) and major targets of Wnt signaling (c-Myc and cyclin D1) is shown. Cells were treated with 10 or 25 μM curcumin at *day 3* for 48 h.

μM curcumin completely blocked differentiation (Fig. 1*A*). Extraction and quantification of Oil red O consistently showed that the lipid accumulation resulting from adipocyte differentiation was significantly reduced in the presence of curcumin (Fig. 1*B*). Differentiation-associated lipid accumulation was accompanied by induction of the master adipogenic transcription factors C/EBPα and PPARγ, and curcumin inhibited the induction of these transcription factors (Fig. 1*C*). Curcumin also suppressed the induction of SREBP-1 and FASN, factors that are downstream of C/EBPα and PPARγ (Fig. 1*C*). These results suggest that curcumin inhibits adipogenic differentiation via downregulation of C/EBPα and PPARγ.

Curcumin attenuates MAPK phosphorylation. To determine whether curcumin inhibits adipogenesis via MAPKs, 3T3-L1 cells were treated with curcumin, and changes in MAPK expression were examined. As shown in Fig. 2, adipocyte differentiation was associated with an increase in phosphorylation of the MAPKs ERK1/2, c-Jun NH_2-terminal kinase (JNK), and p38. Phosphorylated ERK1/2 induces the key adipogenic transcription factor PPARγ via the PI3/Akt pathway (5). JNK participates in oxidative stress and inflammation in adipose tissue and adipocytes (7, 21) and also mediates insulin resistance in 3T3-L1 cells (32). The stress-activated MAPK, p38, is necessary for full differentiation of preadipocytes into adipocytes (11). Interestingly, curcumin treatment abolished the induction of ERK1/2, JNK, and p38 phosphorylation.

Curcumin induces β-catenin translocation. To explore the possible mechanisms by which curcumin inhibits adipogenic differentiation, we analyzed the effect of 25 μM curcumin on β-catenin localization in 3T3-L1 cells. β-Catenin is a key protein that translocates to the nucleus during activation of Wnt signaling. Immunofluorescence labeling revealed that in undifferentiated 3T3-L1 cells, β-catenin was distributed throughout the cytoplasm and nuclei (Fig. 3*A*, *left*). However, in differentiated adipocytes, β-catenin was localized mainly in the cytoplasm (Fig. 3*A*, *middle*). Importantly, treatment of these cells with curcumin restored the nuclear localization of β-catenin (Fig. 3*A*, *right*). Western blot analysis of cytosolic and nuclear fractions was also performed to investigate the effect of curcumin (1–25 μM) on β-catenin localization in 3T3-L1 cells. As shown in Fig. 3*B*, Western blot analysis revealed that differentiation was associated with a decrease in both nuclear and cytosolic β-catenin levels. Thus consistent with immunofluorescence data, curcumin increased nuclear β-catenin levels.

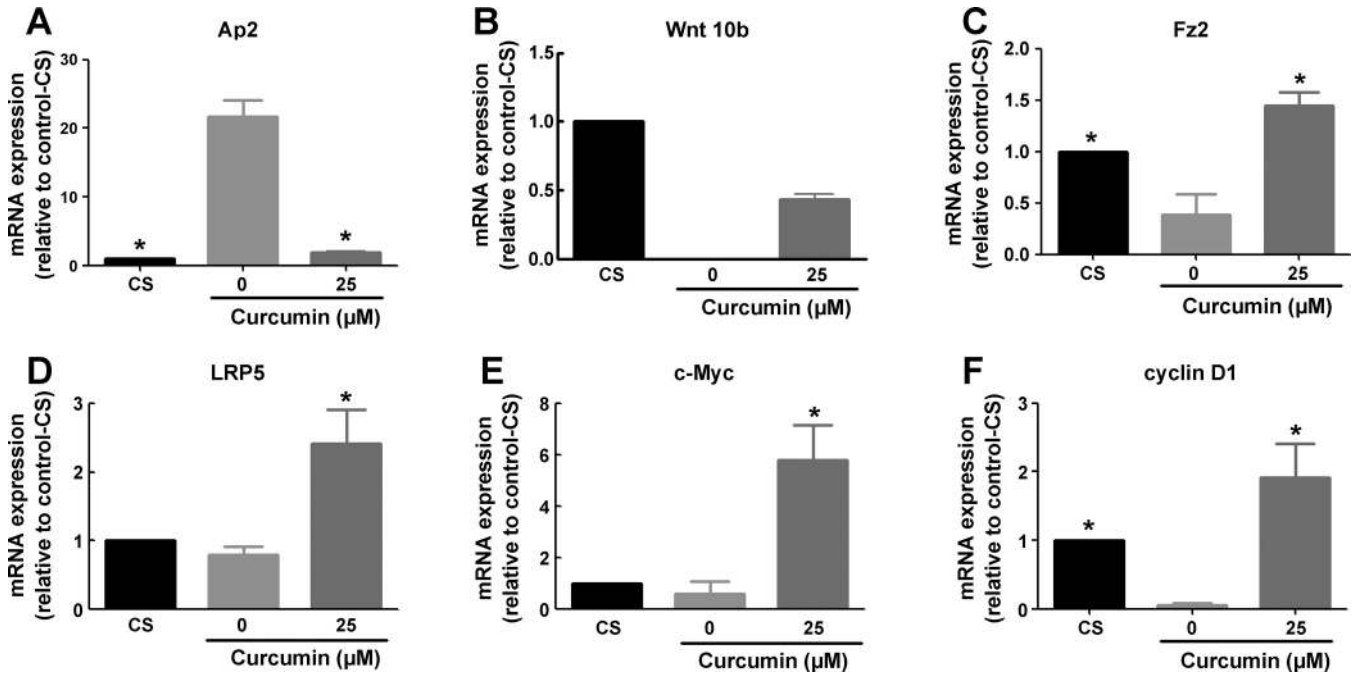

Fig. 5. Effect of curcumin on the genetic expression of Wnt signaling molecules and their targets. Real-time RT-PCR analysis of Wnt signaling molecules (*A–D*) and their targets (*E* and *F*). Cells were treated with 25 μM curcumin at *day 3* for 48 h. Relative mRNA expression was normalized to β-actin expression. *$P < 0.05$ vs. control.

Curcumin activates Wnt signaling. In the absence of Wnt, cellular levels of free β-catenin are tightly controlled by a destructive multiprotein complex that includes Axin, GSK-3, and CK1α. This complex interacts with and phosphorylates β-catenin, rendering it susceptible to degradation (36). Upon stimulation of 3T3-L1 cell differentiation, the expression of GSK-3β, CK1α, and Axin was increased (Fig. 4). This result is consistent with Wnt signaling suppression, which leads to GSK-3β phosphorylation of β-catenin and ubiquitin-mediated degradation of β-catenin in differentiated adipocytes. Importantly, curcumin inhibited differentiation-induced expression of GSK-3β, CK1α, and Axin (Fig. 4). Because the activities of GSK-3 and Axin are inhibited when Wnt signaling is active, these data suggest that curcumin activates Wnt signaling in 3T3-L1 cells. Consistent with this idea, curcumin increased the expression of c-Myc and cyclin D1, the major targets of Wnt (Fig. 4).

Next, we determined the effect of curcumin on the expression of genes related to Wnt signaling. Curcumin markedly inhibited the mRNA expression of Ap2, a marker of mature adipocytes, showing that curcumin effectively inhibited adipocyte differentiation (Fig. 5*A*). Wnt10b activates the canonical Wnt signaling pathway and blocks adipogenesis, making it the best candidate for the endogenous inhibitory Wnt signal. Wnt10b mRNA expression is largely suppressed by induction of differentiation (40). Accordingly, we found that Wnt10b mRNA expression was almost completely suppressed in differentiated adipocytes. Moreover, treatment of 3T3-L1 cells with curcumin increased Wnt10b mRNA expression during exposure to adipogenesis inducers (Fig. 5*B*). Curcumin also increased the mRNA expression of the direct Wnt receptor Fz2 (Fig. 5*C*) and its coreceptor LRP5 (Fig. 5*D*). Finally, we analyzed the mRNA expression of c-Myc and cyclin D1, which are both known targets of Wnt/β-catenin signaling. The expression of c-Myc and cyclin D1 mRNA was reduced in differentiated adipocytes. However, curcumin increased the mRNA expression of these factors (Fig. 5, *E* and *F*).

To determine whether curcumin inhibits adipogenesis during extracellular Wnt inactivation, we exposed preadipocytes to 100 nM secreted frizzled-related protein 2 (sFRP-2), with fresh sFRP-2 added every 2 days. Ten days after the initial treatment, more than 60% of preadipocytes exposed to sFPR-2 had differentiated. Curcumin treatment effectively reversed sFRP-2-induced adipogenesis (Fig. 6, *A* and *B*).

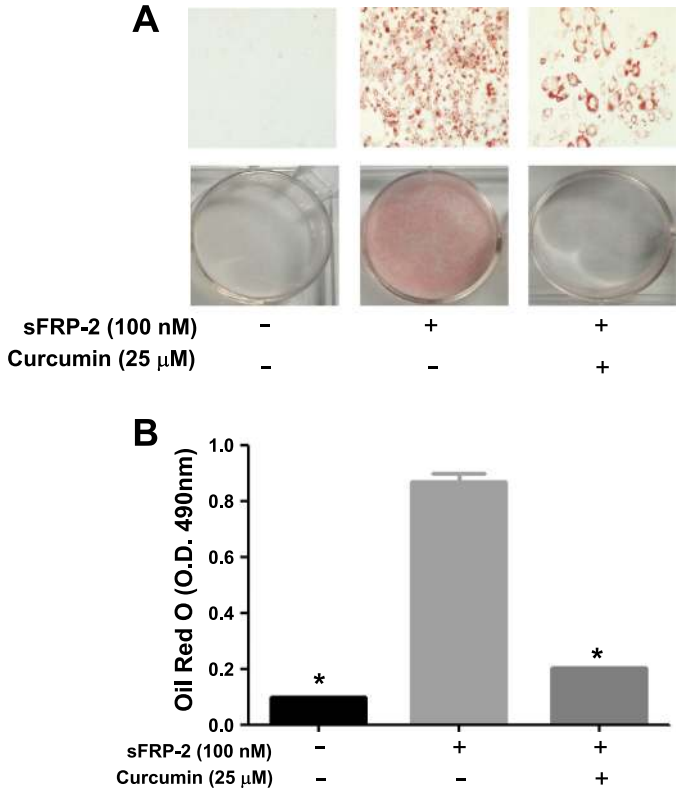

Fig. 6. Effect of curcumin on spontaneous differentiation of preadipocytes exposed to the soluble Wnt inhibitor sFRP-2. *A*: Oil red O staining of 3T3-L1 cells that were exposed to sFRP-2 for 10 days with or without curcumin for the first 2 days. 3T3-L1 cells were grown in 10% CS. The cells were cultured in the presence of 100 nM sFRP-2 for 10 days with or without 25 μM curcumin. Wnt inhibitors were added with the medium changes every 2 days. Curcumin was added only for the first 2 days. Nondifferentiated control cells (*left*) were incubated in DMEM with 10% CS. Representative photomicrographs (×200) are shown for each treatment group. *B*: quantitative analysis of adipocyte differentiation, as assessed by spectrophotometric measurement of Oil Red O-stained adipocytes. *$P < 0.05$ vs. sFRP-2-treated group.

DISCUSSION

Development of obesity is characterized by the growth of adipose tissue mass. Growth in both the size and numbers of adipocytes is essential for lipid accumulation in fat tissue. Although a controlled diet and high level of physical activity are recommended therapies for obesity (43, 47), inhibition of adipogenesis, including reducing adipocyte numbers and the fat content of adipocytes, is an additional strategy for reducing obesity. Curcumin may be a candidate for this purpose, as we have found that it decreases differentiation of preadipocytes. When used at a concentration of 25 μM, curcumin clearly suppressed the differentiation of 3T3-L1 cells grown in adipogenesis-inducing medium. Importantly, our data suggest that the inhibitory effect of curcumin on preadipocyte differentiation is mediated by the activation of canonical Wnt/β-catenin signaling.

Here we show that curcumin effectively blocked adipocyte differentiation by suppressing induction of adipogenic transcription factors such as C/EBPα and PPARγ. PPARγ and C/EBPα, which are mainly found in adipose tissue, are the key transcription factors regulating adipogenesis and lipogenesis (51). The finding that curcumin suppresses differentiation of 3T3-L1 adipocytes has also been recently reported by another group (8). The genetic program of adipogenesis has been extensively studied in vitro using preadipocyte lines. These studies have shown that, after induction of differentiation, C/EBPβ and C/EBPδ are rapidly and transiently induced. These transcription factors then activate expression of both C/EBPα and PPARγ, which then stimulate one another's expression via a positive feedback loop (39). As clearly shown here, curcumin reduces the expression of not only C/EBPα and PPARγ, but also their downstream factors, SREBP-1 and FASN.

Dephosphorylation of MAPKs was observed in adipocytes differentiated in the presence of curcumin. The ability of curcumin to modulate the MAPK signaling pathway may explain, at least partly, the anti-adipogenic effects of this compound. Curcumin is known to inhibit MAPK activation (25). For example, curcumin attenuates experimental colitis by reducing the activity of p38 MAPK (42). In addition, gene

expression profiling has shown that curcumin downregulates the expression of MEKK4, MKK4, and JNK (53), suggesting that it inhibits MAPK signaling. Therefore, decreased MAPK phosphorylation may be one of the mechanisms underlying the anti-adipogenic activity of curcumin.

We have observed that curcumin activates Wnt/β-catenin signaling in differentiated adipocytes. Considerable evidence now suggests that Wnt/β-catenin signaling is an important regulator of adipocytes and plays a critical role in adipogenesis. Wnt proteins inhibit adipocyte differentiation through both β-catenin-dependent and -independent mechanisms (24, 38). We found that, in differentiated adipocytes, β-catenin was mainly located in the cytoplasm, indicating that the Wnt/β-catenin pathway was inhibited. However, treatment of cells with curcumin induced the translocation of β-catenin to the nucleus. In the nucleus, unphosphorylated β-catenin forms a transcription complex upon binding to TCF4. This binding modulates the expression of target genes, including cyclin D1, c-Myc, and c-jun. In the absence of Wnt signaling, cytoplasmic β-catenin is targeted for proteasomal degradation by a heteromeric protein complex that includes Axin, GSK-3β, and CK1 (18, 19, 41), and curcumin inhibited the expression of these inhibitory proteins. This in turn, activated Wnt/β-catenin signaling in curcumin-treated 3T3-L1 cells. Recently, it has been reported that GSK-3β plays a critical role in JNK-induced suppression of Wnt/β-catenin signaling (16, 17). Therefore, it has been suggested that curcumin downregulates JNK phosphorylation by inhibiting GSK-3β. Axin, a negative regulator of canonical Wnt signaling, is a scaffold protein in the β-catenin destruction complex, which promotes the degradation of β-catenin. Axin stimulates the phosphorylation of β-catenin by GSK3β (15, 30). The levels of Axin decrease following exposure of cells to Wnt (49, 52). Because curcumin increased Wnt10b gene expression in our experiment, we postulate that the increase in Wnt10b inhibited Axin.

In the presence of adipogenesis-inducing stimuli, curcumin enhanced the expression of c-Myc and cyclin D1, which are well-known downstream target genes of β-catenin (14, 46). Tumor necrosis factor (TNF)-α-induced inhibition of adipogenesis is associated with sustained expression of c-Myc (33), and forced expression of c-Myc prevents adipogenesis by inhibiting the expression of PPARα (9). Moreover, cyclin D1 has been shown to inhibit adipogenesis by inhibiting the expression of C/EBPα (10). Therefore, Wnt signaling was downregulated during differentiation (40). Candidate proteins involved in differentiation include known targets of the Wnt signaling pathway, such as cyclin D1 (45) and c-Myc (14). In contrast, several studies have demonstrated that curcumin decreases cyclin D1 and c-Myc expression in cancer cells. The anti-cancer effect of curcumin was correlated with antagonizing Wnt/β-catenin in cancer cells (20, 28, 37, 44).

Wnt10b is highly expressed in preadipocytes and declines rapidly after induction of differentiation (2, 40). Overexpression of Wnt10b in 3T3-L1 preadipocytes stabilizes β-catenin and blocks adipogenesis. Moreover, the addition of Win10b anti-sera to 3T3-L1 medium promotes adipocyte differentiation (26). Consistent with these findings, we found that Wnt10b mRNA was absent in differentiated adipocytes and that curcumin treatment restored Wnt10b mRNA in these cells. Intriguingly, we observed that an early 48-h exposure to curcumin effectively blocked adipogenesis. This response may be explained by the finding that adipogenesis programs are sensitive to inhibition by Wnt signaling during the early stages of differentiation (2).

The inhibitory Wnt signals on adipogenesis are mediated by direct Fz (Fz1, Fz2, or Fz5) receptors and LRP coreceptors (LRP4 and LRP5), which is consistent with the observation that expression of these Wnt receptors is high in preadipocytes and declines upon induction of differentiation (2). We found that curcumin greatly increased the mRNA expression of Fz2 and LRP5. Moreover, we also demonstrated that curcumin inhibited adipogenesis even when extracellular Wnts were suppressed by sFRP-2. sFRPs, members of the Wnt antagonist family, possess a cysteine-rich domain similar to Fz and act either by binding directly to Wnts or by dimerizing with Fz to form nonfunctional complexes (23). sFRP-2, a member of this family, is reported to be a potential suppressor of Wnt (35). These results show that activation of the Wnt/β-catenin pathway by curcumin inhibits adipogenesis in 3T3-L1 cells.

In summary, curcumin, the major polyphenol found in turmeric, effectively inhibits preadipocyte differentiation. Dephosphorylation of MAPKs may participate in the anti-adipogenic effects of curcumin. Curcumin also inhibited the induction of adipogenic transcription factors such as PPARγ and C/EBPα. This study is the first to demonstrate that the Wnt/β-catenin signaling pathway plays a crucial role in curcumin-induced suppression of adipogenesis.

GRANTS

This study was supported by the Bio-Food Research Project from the Korea Science and Engineering Foundation (KOSEF), National Platform Technology Project from Ministry of Knowledge Economy in Korea, and the Korea Food Research Institute.

DISCLOSURES

No conflicts of interest are declared by the author(s).

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