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Bisindoylmaleimide I suppresses adipocyte differentiation through stabilization of intracellular β-catenin protein

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Abstract

The Wnt/β-catenin signaling pathway plays important roles in cell differentiation. Activation of this pathway, likely by Wnt-10b, has been shown to inhibit adipogenesis in cultured 3T3-L1 preadipocytes and mice. Here we revealed that bisindoylmaleimide I (BIM), which is widely used as a specific inhibitor of protein kinase C (PKC), inhibits adipocyte differentiation through activation of the Wnt/β-catenin signaling pathway. BIM increased β-catenin responsive transcription (CRT) and up-regulated intracellular β-catenin levels in HEK293 cells and 3T3-L1 preadipocytes. BIM significantly decreased intracellular lipid accumulation and reduced expression of important adipocyte marker genes including peroxisome-proliferator-activated receptor γ (PPARγ) and CAATT enhancer-binding protein α (C/EBPα) in 3T3-L1 preadipocytes. Taken together, our findings indicate that BIM inhibits adipogenesis by increasing the stability of β-catenin protein in 3T3-L1 preadipocyte cells.

Keywords: 3T3-L1 preadipocytes; Adipogenesis; BIM; C/EBPα; PPARγ; Wnt/β-catenin signaling pathway

Cellular progression from preadipocyte to adipocyte, or adipogenesis, is a complex process involving coordinated changes in morphology, hormone sensitivity, and gene expression, and is regulated by a balance of local and endocrine factors [1,2]. The molecular mechanisms that govern adipogenesis have been well investigated using immortalized preadipocyte cell lines such as 3T3-L1 and 3T3-F442A [3–7]. In response to adipogenic stimuli, CAATT enhancer-binding protein β (C/EBPβ), and C/EBPδ are rapidly and transiently up-regulated during the early stages of adipogenesis, which subsequently promotes the expression of the key adipogenic transcription factors, C/EBPα, and peroxisome-proliferator-activated receptor γ (PPARγ). These factors synergistically induce the expression of various genes that are required for the adipocyte phenotype.

Wnts are a family of secreted glycoproteins that control diverse developmental processes and homeostasis [8,9]. The interaction of Wnts with Frizzled (Fz) receptors and low-density lipoprotein receptor-related protein (LRP) coreceptors activates several signaling pathways, such as the Wnt/β-catenin pathway, the planar cell polarity pathway, and the Wnt/Ca^{2+} pathway [10–12]. Best understood among these is the Wnt/β-catenin pathway, which is controlled by cytoplasmic β-catenin levels [10]. In the absence of a Wnt signal, cytoplasmic β-catenin is phosphorylated by casein kinase 1 (CK1) and glycogen synthase kinase-3β (GSK-3β) in a multi-protein complex containing adenomatous polyposis coli (APC) and Axin, resulting in the degradation of β-catenin via an ubiquitin-dependent mechanism [13]. Activation of the receptor by its Wnt ligands inhibits GSK-3β, leading to the stabilization of β-catenin in the

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cytoplasm [14]. This is followed by translocation to the nucleus, where β-catenin forms a complex with members of the T cell factor/lymphocyte enhancer factor (TCF/LEF) transcription factor families, which activates the expression of Wnt/β-catenin responsive genes [15–18].

The Wnt/β-catenin signaling pathway has been reported to inhibit adipogenic conversion of 3T3-L1 preadipocytes through the down-regulation of C/EBPα and PPARγ [19,20]. Ectopic expression of Wnt1 or Wnt-10b in 3T3-L1 preadipocytes induces the accumulation of cytoplasmic β-catenin and suppresses adipocyte differentiation [21]. In addition, pharmacological treatments (e.g., LiCl or CHIR99021) that mimic activation of the Wnt/β-catenin signaling pathway also repress adipogenesis [22]. In contrast, inhibiting the Wnt/β-catenin signaling pathway by over-expressing Axin or dominant-negative Tcf-4 in 3T3-L1 leads to spontaneous adipogenesis. Here we report that BIM mimics activation of the Wnt/β-catenin signaling pathway in preadipocytes, and that BIM induces accumulation of cytoplasmic β-catenin while suppressing adipocyte differentiation by blocking the expression of C/EBPα and PPARγ.

Materials and methods

Cell culture, plasmid transfection, and luciferase assay. L cells that secrete Wnt3a, HEK293 cells, and 3T3-L1 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 120 μg/ml penicillin, and 200 μg/ml streptomycin. The HEK293 reporter and control cell line was established as previously described [23]. Wnt3a conditioned medium (Wnt3a CM) was prepared by culturing Wnt3a-secreting L cells in DMEM with 10% FBS for 4 days. The medium was harvested and sterilized using a 0.22-μm filter. Fresh medium was added, and the cells were cultured for 3 additional days. The medium was then collected and combined with the previous medium. The pTOPFlash and pFOPFlash plasmids were obtained from Upstate Biotechnology (Lake Placid, NY, USA) and pCMV-RL were purchased from Promega (Madison, WI, USA). The transfections were carried out with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Luciferase assays were performed using the Dual Luciferase Assay Kit (Promega).

Adipocyte differentiation. 3T3-L1 preadipocytes were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C. After 2 days of confluence (day 0), cells were incubated for 2 days in DMEM supplemented with 10% FBS, 0.5 mM of 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 10 μg/ml insulin. Thereafter, medium was replaced every other day with DMEM containing 10% FBS and 10 μg/ml insulin. BIM were treated into cells and supplemented at 2-day intervals when the culture medium was changed.

Oil Red O staining. 3T3-L1 preadipocytes were rinsed in PBS and fixed in 3.7% paraformaldehyde for 1 h at 4 °C. Briefly, plates were stained with 1% Oil Red O in 60% isopropanol for 10 min. The stain was then differentiated with 60% isopropanol and plates were washed several times in distilled water prior to visualization under a phase-contrast microscope. Quantitative analysis of adipocyte differentiation was performed by measuring the OD 520 nm of the Oil Red O-stained adipocytes eluted with isopropanol and Igepal CA 40.

Western blotting. The cytosolic fraction was prepared as previously described [24]. Proteins were separated by 4–12% gradient SDS–PAGE (Invitrogen) and transferred to a nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ, USA). The membranes were blocked with 5% nonfat milk and probed with anti-β-catenin (BD Transduction Laboratories, Lexington, KY, USA), anti-C/EBPα (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-PPARγ (Santa Cruz Biotechnology), and anti-actin antibodies (Cell Signaling Technology, Beverly, MA, USA). After washing, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG (Santa Cruz Biotechnology), and the bands were visualized using the ECL system (Santa Cruz Biotechnology).

RNA extraction and semiquantitative RT-PCR. Total RNA was isolated with TRIzol reagent (Invitrogen) in accordance with the manufacturer's instructions. cDNA synthesis, reverse transcription, and PCR were performed as described previously [25]. Primers for each gene were as follows: for C/EBPα (sense, 5′-AGGTGCTGGAGTTGACCAGT-3′; antisense, 5′-CAGCCTAGAGATCCAGCGAC-3′), PPARγ (sense, 5′-GAGCATG GT GCCTTCGCTGAT-3′, antisense, 5′-CAACCAT TGGGTCACCTCTTG-3′), β-catenin (sense, 5′-GGATTCTGGAATCC ATTCTGG-3′, antisense, 5′-TCTGAGCCCTAGTCATTGCAT-3′), and aP2 (sense, 5′-TACATGAAAGAAGTGGGAGTG-3′, antisense, 5′-GGTGATTTCATCGAATTCCAC-3′). The amplified DNA was separated on 1.2% agarose gels and stained with ethidium bromide.

Results

BIM activates the Wnt/β-catenin signaling pathway

We recently demonstrated that protein kinase C (PKC) phosphorylates Ser33/37 of β-catenin, resulting in the promotion of β-catenin degradation [26]. In addition, small interference RNA (siRNA)-mediated knockdown of PKCα inhibits Ser33/37 phosphorylation of β-catenin and induces the accumulation of β-catenin in HEK293 cells [26]. Thus, we postulated that a pharmacological inhibitor of PKC such as bisindoylmaleimide I (BIM) would mimic activation of the Wnt/β-catenin signaling pathway. To test this hypothesis, HEK293 reporter cells that were stably transfected with TOPFlash, a synthetic Tcf/β-catenin-dependent luciferase reporter, and human Frizzled-1 (hFz-1) expression plasmid were incubated with BIM and monitored for TOPFlash reporter activity using a microplate reader. As shown in Fig. 1A, this compound up-regulated β-catenin response transcription (CRT) in a dose-dependent manner whereas it did not affect FOPflash reporter activity in HEK control cells, indicating that BIM is a specific activator of the Wnt/β-catenin signaling pathway.

Within the Wnt/β-catenin signaling pathway, CRT is largely dependent on the level of cytoplasmic β-catenin, which is regulated by an ubiquitin-dependent proteasome pathway [10]. To investigate the effect of BIM on the level of β-catenin, we analyzed the amount of cytoplasmic β-catenin by Western blotting with anti-β-catenin antibody. Interestingly, treatment with BIM resulted in increased β-catenin levels in cytoplasm, which is consistent with its effect on CRT (Fig. 1B). However, the β-catenin mRNA level did not change in response to different concentrations of BIM in HEK293 reporter cells (Fig. 1C). These results suggest that BIM activates the Wnt/β-catenin signaling pathway via the up-regulation of β-catenin protein stability.

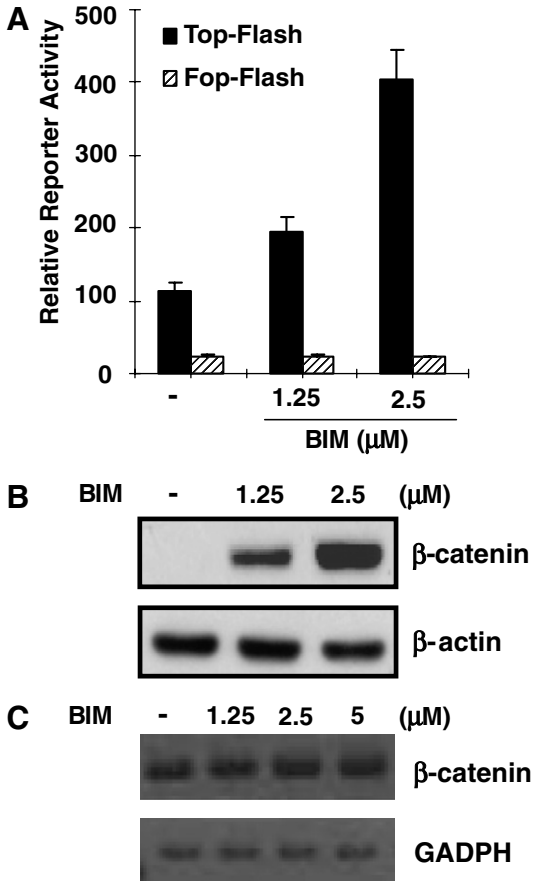

Fig. 1. BIM mimics activation of the Wnt/β-catenin signaling pathway in HEK293 reporter cells. (A) Dose-dependent response for CRT activation with increasing concentrations of BIM. HEK293 reporter and control cells were incubated with BIM (1.25, 2.5 μM) for 15 h and luciferase activity was determined. The results are shown as the average of three experiments; the bars indicate standard deviations. (B) Cytosolic proteins were prepared from HEK293 cells treated with either vehicle (DMSO) or BIM (1.25, 2.5 μM) for 15 h and then subjected to Western blotting with β-catenin antibody. The blots were re-probed with anti-actin antibody as a loading control. (C) Semiquantitative RT-PCR for β-catenin and GAPDH was performed with total RNA prepared from HEK293 reporter cells treated with the vehicle (DMSO) or BIM (1.25, 2.5, 5 μM) for 15 h.

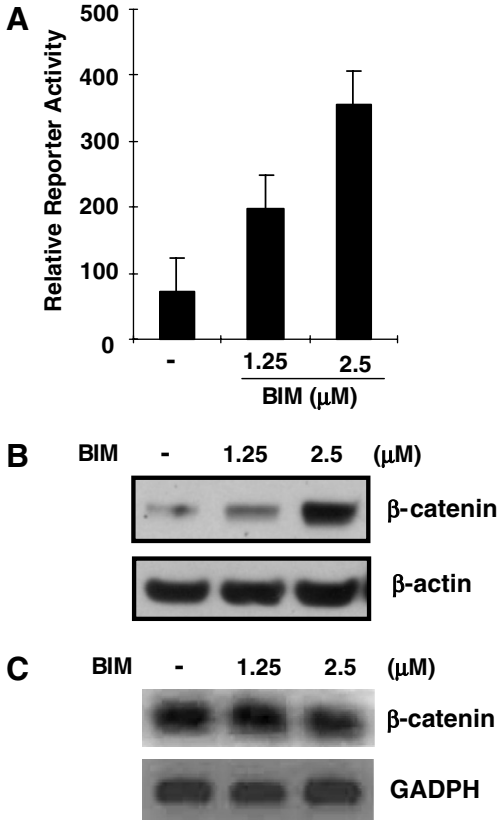

Fig. 2. BIM mimics activation of the Wnt/β-catenin signaling pathway in 3T3-L1 preadipocytes. (A) 3T3-L1 preadipocytes were cotransfected with TOPFlash and pCMV-RL plasmids and incubated with BIM (1.25, 2.5 μM) for 15 h. Luciferase activities were measured 39 h after transfection. Results are the average of three experiments, and the bars indicate standard deviations. (B) Cytosolic proteins were prepared from 3T3-L1 preadipocytes treated with either vehicle (DMSO) or BIM (1.25, 2.5 μM) for 15 h and then subjected to Western blotting with β-catenin antibody. The blots were reprobed with anti-actin antibody as a loading control. (C) Semiquantitative RT-PCR for β-catenin and GAPDH were performed with total RNA prepared from 3T3-L1 preadipocytes treated with the vehicle (DMSO) or BIM (1.25, 2.5 μM) for 15 h.

BIM suppresses adipocyte differentiation through activation of the Wnt/β-catenin signaling pathway

Activation of the Wnt/β-catenin signaling pathway, followed by the accumulation of intracellular β-catenin, greatly suppresses adipocyte differentiation [19,20]. Given that BIM induces the accumulation of cytoplasmic β-catenin in HEK293 reporter cells, we hypothesized that BIM also inhibits the differentiation of preadipocytes to adipocytes. To test this hypothesis, we first examined whether 3T3-L1 preadipocytes are capable of transducing Wnt/β-catenin signaling when treated with BIM. We found that 3T3-L1 preadipocytes treated with BIM indeed showed a consistent and robust, concentration-dependent increase in TOPFlash reporter activity (Fig. 2A). In addition, the intracellular β-catenin level, an indicator of the activation status of Wnt/β-catenin signaling pathway, was also increased by incubation with BIM in 3T3-L1 preadipocytes (Fig. 2B). Finally, the β-catenin mRNA level remained consistently unchanged in response to different concentrations of BIM in 3T3-L1 preadipocytes (Fig. 2C). These results suggest that the Wnt/β-catenin signaling pathway can be activated in response to BIM in 3T3-L1 preadipocytes.

To examine whether activation of the Wnt/β-catenin signaling pathway by BIM inhibits adipogenic differentiation, 3T3-L1 preadipocytes were incubated with increasing amounts of BIM beginning at day 0 of the standard differentiation protocol. As shown in Fig. 3A, treatment with BIM resulted in a concentration-dependent decrease in lipid droplet accumulation induced by stimulator of adipogenesis as assessed by Oil Red O staining (Fig. 3A). Extraction and quantification of Oil Red O further confirmed that MDI-induced adipocyte differentiation was inhibited by BIM (Fig. 3B). These results indicate that activation of

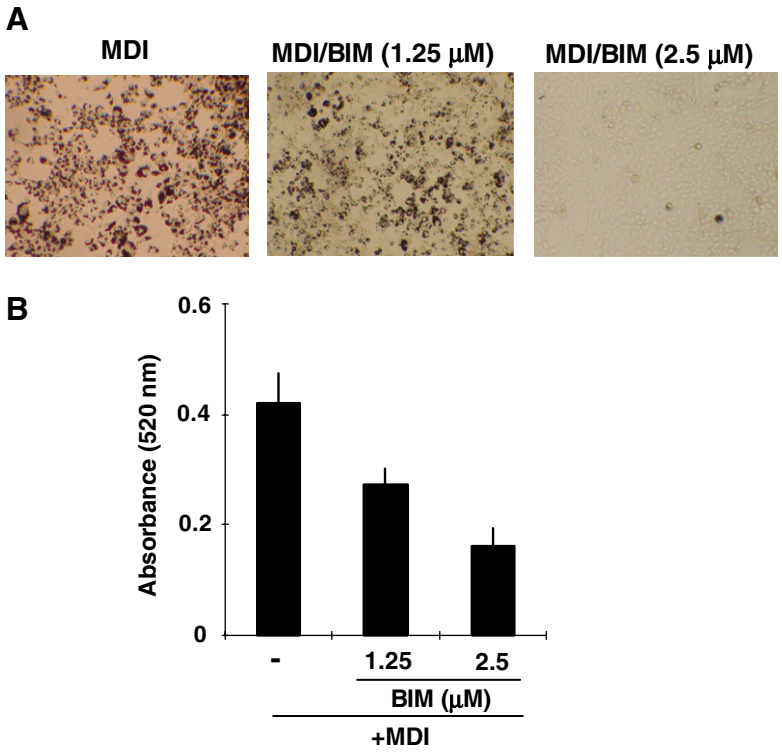

Fig. 3. BIM suppresses preadipocyte differentiation. (A) 3T3-L1 preadipocytes were differentiated into adipocytes as described in Materials and methods. Increasing amounts of BIM (1.25, 2.5 μM) were administered to cells during adipocyte differentiation. One week after induction of differentiation, adipocytes were stained with Oil Red O and photographed. (B) Oil Red O was extracted with 4% Igepal in isopropanol and quantified using spectrophotometry at 520 nm. The results are shown as the average of three experiments; the bars indicate standard deviations.

the Wnt/β-catenin signaling pathway by BIM blocks adipocyte conversion (see Fig. 4).

BIM inhibits adipogenic gene expression

Previous studies have demonstrated that preadipocyte differentiation is accompanied by induction of the master adipogenic transcription factors such as C/EBPα and PPARγ [3–7]. Therefore, we examined the effects of BIM on the expression of C/EBPα and PPARγ. Consistent with the morphological changes observed, the level of C/EBPα and PPARγ was increased by incubation with MDI (Fig. 3A); however, MDI-mediated C/EBPα and PPARγ up-regulation was abolished by the addition of BIM (Fig. 3A). Similar to the protein level results, we observed that mRNA expression of C/EBPα and PPARγ, which had been induced by MDI, was reduced by BIM treatment (Fig. 3B). Moreover, BIM repressed the induction of the adipocyte lipid-binding protein aP2, which is downstream of C/EBPα and PPARγ (Fig. 3B). These results suggest that BIM inhibits adipogenesis by suppressing the expression of C/EBPα and PPARγ.

Discussion

Recent studies have reported that activation of the Wnt/β-catenin signaling pathway inhibits adipogenic differentiation [15–18]. Central to this pathway is the level of cytoplasmic β-catenin that is controlled by ubiquitin-dependent proteolysis [10]. Multiple pathways regulate the degradation of intracellular β-catenin. In the GSK-3β-dependent pathway, β-catenin is phosphorylated by a multi-protein complex GSK-3β, resulting in the promotion of β-catenin degradation through a β-TrCP-dependent mechanism [27,28]. In the Siah-dependent pathway, Siah-1 interacts with the carboxyl terminus of APC, which recruits the ubiquitination complex and leads to the degradation of β-catenin via a GSK-3β- and β-TrCP-independent pathway [29]. Recently, we demonstrated that PKC-mediated β-catenin phosphorylation at Ser33/37 residues negatively regulates the Wnt/β-catenin signaling pathway by promoting β-catenin degradation [26]. In addition, depletion of PKCα using siRNA inhibits Ser33/37 phosphorylation of β-catenin and induces the accumulation of β-catenin protein [26]. Here we showed that BIM, a specific pharmacological inhibitor of PKC, was also able to mimic activation of the Wnt/β-catenin signaling pathway. β-catenin response transcription (CRT) increased dramatically when HEK293 reporter cells and 3T3-L1 preadipocytes were incubated with BIM. Moreover, intracellular β-catenin protein was stabilized by BIM without altering β-catenin mRNA levels. These results further confirm that the PKC pathway negatively regulates the intracellular β-catenin level outside of the Wnt/β-catenin signaling pathway in HEK293 reporter cells and 3T3-L1 preadipocytes.

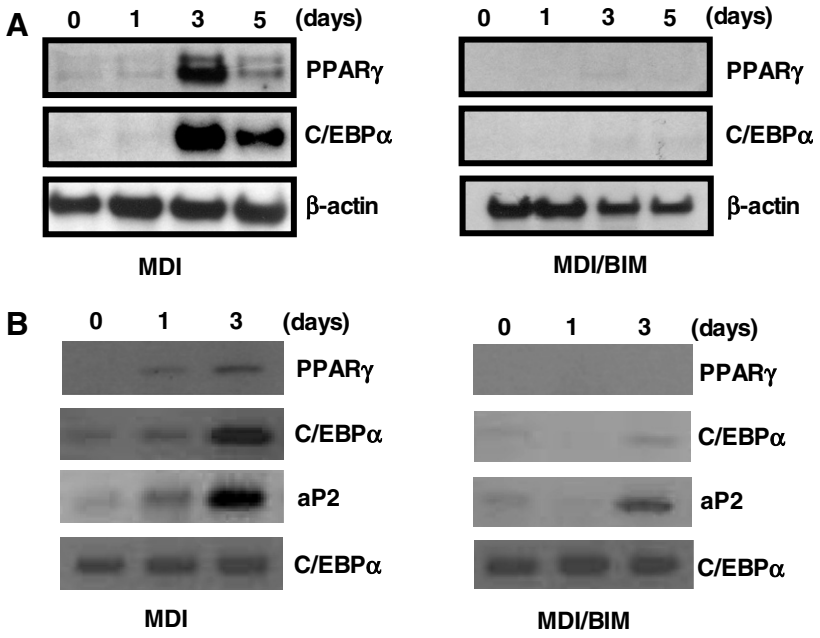

Fig. 4. BIM inhibits the expression of adipogenic factors during adipocyte differentiation. (A) 3T3-L1 preadipocytes were differentiated into adipocytes as described in the Materials and methods. BIM (2.5 μM) was administered to cells during adipocyte differentiation. On the indicated days, total proteins were prepared from 3T3-L1 preadipocytes and then subjected to Western blotting with anti-PPARγ and anti-C/EBPα antibodies. The blots were reprobed with anti-actin antibody as a loading control. (B) Semiquantitative RT-PCR for PPARγ, C/EBPα, aP2, and GAPDH was performed with total RNA prepared from 3T3-L1 preadipocytes treated with BIM (2.5 μM) on the indicated days.

The PKC family plays important roles in various biological functions, such as proliferation, differentiation, cell migration, and apoptosis [30–32]. Several studies have suggested that the PKC pathway is involved in the regulation of adipogenesis [33,34], and it was recently shown that inhibiting the PKC pathway with pharmacological inhibitors, such as Ro318220 and Go6976, inhibits adipocyte differentiation [35]; however, the precise mechanism remains largely unknown. We demonstrated that the up-regulation of CRT and stabilization of intracellular β-catenin, which indicates activation of the Wnt/β-catenin signaling pathway, were induced by treatment with BIM, and thereby suppressed adipogenic differentiation in 3T3-L1 preadipocytes. These results suggest that inhibiting the PKC pathway suppresses adipogenesis via activation of the Wnt/β-catenin signaling pathway.

In summary, we showed that BIM, which was able to stabilize intracellular β-catenin protein levels, is an inhibitor of adipogenesis. Mutations in the Wnt-10b gene have recently been implicated in human obesity [36], and LRP5 polymorphisms have been shown to be significantly associated with an obese phenotype [37]. Taken together, our findings may facilitate the development of new therapeutics for obesity and its associated disorders.

Acknowledgments

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References

[1] B.M. Spiegelman, J.S. Flier, Adipogenesis and obesity: rounding out the big picture, Cell 87 (1997) 212–218.
[2] O.A. MacDougald, S. Mandrup, Adipogenesis: forces that tip the scales, Trends Endocrionol. Metab. 13 (2002) 5–11.
[3] S.M. Rangwala, M.A. Lazar, Transcriptional control of adipogenesis, Annu. Rev. Nutr. 20 (2000) 535–559.
[4] E.D. Rosen, B.M. Spiegelman, Molecular regulation of adipogenesis, Annu. Rev. Cell Dev. Biol. 16 (2000) 145–171.
[5] E.D. Rosen, C.J. Walkey, P. Puigserver, B.M. Spiegelman, Transcriptional regulation of adipogenesis, Genes Dev. 14 (2000) 1293–1307.
[6] R.P. Brun, J.B. Kim, E. Hu, B.M. Spiegelman, Peroxisome proliferators-activated receptor gamma and the control of adipogenesis, Curr. Opin. Lipidol. 8 (1977) 212–218.
[7] R.J. Christy, K.H. Kaestner, D.E. Geiman, M.D. Lane, CCAAT/enhancer binding protein gene promoter: binding of nuclear factors during differentiation of 3T3-L1 preadipocytes, Proc. Natl. Acad. Sci. USA 88 (1991) 2593–2597.
[8] A. Wodarz, R. Nusse, Mechanisms of Wnt signaling in development, Annu. Rev. Cell Dev. Biol. 14 (1998) 59–88.
[9] P. Polakis, Wnt signaling and cancer, Genes Dev. 14 (2000) 1837–1851.
[10] R.H. Giles, J.H. vsn Es, H. Clevers, Caught up in a Wnt storm: Wnt signaling in cancer, Biochim. Biophys. Acta 1653 (2003) 1–24.
[11] M. Kuhl, L.C. Sheldahl, M. Park, J.R. Miller, R.T. Moon, The Wnt/Ca^{2+} pathway: a new vertebrate Wnt signaling pathway takes shape, Trends Genet. 16 (2000) 279–283.
[12] R. Winklbauer, A. Medina, R.K. Swain, H. Steinbeisser, Frizzled-7 signalling controls tissue separation during *Xenopus* gastrulation, Nature 413 (2001) 856–860.
[13] C. Liu, Y. Li, M. Semenov, C. Han, G.H. Baeg, Y. Tan, Z. Zhang, X. Lin, X. He, Control of β-catenin phosphorylation/degradation by a dual-kinase mechanism, Cell 108 (2002) 837–847.
[14] E. Lee, A. Salic, R. Kruger, R. Heinrich, M.W. Kirschner, The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway, PLoS Biol. 1 (2003) 116–132.

[15] O. Tetsu, F. McCormick, β-Catenin regulates expression of cyclin D1 in colon carcinoma cells, Nature 398 (1999) 422–426.
[16] T.C. He, A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da Costa, P.J. Morin, B. Vogelstein, K.W. Kinzler, Identification of c-MYC as a target of the APC pathway, Science 281 (1998) 1509–1512.
[17] M. Takahashi, T. Tsunoda, M. Seiki, Y. Nakamura, Y. Furukawa, Identification of membrane-type metrix metalloproteinase-7 as a target of the β-catenin/TCF-4 complex in human colorectal cancers, Oncogene 21 (2002) 5861–5867.
[18] T.C. He, T.A. Chan, B. Vogelstein, K.W. Kinzler, PPARδ is an APC-regulated target of nonsteroidal anti-inflammatory drugs, Cell 99 (1999) 335–345.
[19] C.N. Bennett, C.L. Hodge, O.A. MacDougald, J. Schwartz, Role of Wnt 10b and C/EBPα in spontaneous adipogenesis of 243 cells, Biochem. Biophys. Res. Commun. 302 (2003) 12–16.
[20] C. Christodoulides, M. Laudes, W.P. Cawthorn, S. Schinner, M. Soos, S. O'Rahilly, J.K. Sethi, A. Vidal-Puig, The Wnt antagonist Dickkopf-1 and its receptors are coordinately regulated during early human adipogenesis, J. Cell Sci. 119 (2006) 2613–2620.
[21] S.E. Ross, N. Hemati, K.A. Longo, C.N. Bennett, P.C. Lucas, R.L. Erickson, O.A. MacDougald, Inhibition of adipogenesis by Wnt signaling, Science 289 (2000) 950–953.
[22] C.N. Bennett, S.E. Ross, K.A. Longo, L. Bajnok, N. Hemati, K.W. Jhonson, S.D. Harrison, O.A. MacDougald, Regulation of Wnt signaling during adipogenesis, J. Biol. Chem. 277 (2002) 30998–31004.
[23] S. Park, J. Gwak, M. Cho, T. Song, J. Won, D.E. Kim, J.G. Shin, S. Oh, Hexachlorophene inhibits Wnt/β-catenin pathway by promoting Siah-mediated β-catenin degradation, Mol. Pharmacol. 70 (2006) 960–966.
[24] J.D. Dignam, R.M. Lebovitz, R.G. Roeder, Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei, Nucleic Acids Res. 11 (1983) 1475–1489.
[25] G.Y. Song, J.H. Lee, M. Cho, B.S. Park, D.E. Kim, S. Oh, Decursin suppresses human androgen-independent PC3 prostate cancer cell proliferation by promoting the degradation of β-catenin, Mol. Pharmacol. 72 (2007) 1599–1606.
[26] J. Gwak, M. Cho, S.J. Gong, J. Won, D.E. Kim, E.Y. Kim, S.S. Lee, M. Kim, T.K. Kim, J.G. Shin, S. Oh, Protein-kinase-C-mediated β-catenin phosphorylation negatively regulates the Wnt/β-catenin pathway, J. Cell Sci. 119 (2006) 4702–4709.
[27] M.J. Hart, R. del los Santos, I.N. Albert, B. Rubinfeld, P. Polakis, Downregulation of β-catenin by human Axin and its association with the APC tumor suppressor, β-catenin and GSK3β, Curr. Biol. 8 (1998) 573–581.
[28] H. Aberle, A. Bauer, J. Stappert, A. Kispert, R. Kemler, β-Catenin is a target for the ubiquitin-proteasome pathway, EMBO J. 16 (1997) 3797–3804.
[29] J. Liu, J. Stevens, C.A. Rote, H.J. Yost, Y. Hu, K.L. Neufeld, R.L. White, N. Matsunami, Siah-1 mediates a novel β-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein, Mol. Cell 7 (2001) 927–936.
[30] M.J. Clemens, I. Trayner, J. Menaya, The role of protein kinase C isoenzymes in the regulation of cell proliferation and differentiation, J. Cell Sci. 103 (1992) 881–887.
[31] G.I. Gallicano, M.C. Yousef, D.G. Capco, PKC-a pivotal regulator of early development, Bioassay 19 (1997) 29–36.
[32] M.F. McCarty, Up-regulation of intracellular signaling pathways may play a central pathogenic role in hypertension, atherogenesis, insulin-resistance and cancer promotion-the 'PKC syndrome', Med. Hypotheses 46 (1996) 113–120.
[33] I. Flemings, S.J. MacKenzie, R.G. Vernon, N.G. Anderson, M.D. Houslay, E. Kilgour, Protein kinase C isoforms play differential roles in the regulation of adipocyte differentiation, Biochem. J. 333 (1998) 719–727.
[34] D. Lacasa, B. Agli, Y. Giudicelli, Zeta PKC in rat preadipocytes: modulation by insulin and serum mitogenic factors and possible role in adipogenesis, Biochem. Biophys. Res. Commun. 217 (1995) 123–130.
[35] Y. Zhou, D. Wang, F. Li, J. Shi, J. Song, Different roles of protein kinase C-b1 and d in the regulation of adipocyte differentiation, Int. J. Biochem. Cell Biol. 38 (2006) 2151–2163.
[36] C. Christodoulides, A. Scarda, M. Granzotto, G. Milan, E. Dalla Nora, J. Keogh, G. De perogola, H. Stirling, N. Pannacciulli, J.K. Sethi, G. Federspil, I. Vidal-Puig, I.S. Farooqi, S. O'Rahilly, R. Vettor, Wnt10b mutations in human obesity, Diabetologia 49 (2006) 678–684.
[37] Y.D. Guo, H. Xiong, H. Shen, I. Zhao, P. Xiao, Y. Guo, W. Wang, T. Yang, R. Robert, H. Deng, Polymorphism of the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with obesity phenotype in a large family-based association study, J. Med. Genet. 43 (2006) 798–803.