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# Tetrahydropalmatine promotes myoblast differentiation through activation of p38MAPK and MyoD

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ABSTRACT

Myoblast differentiation is fundamental to the development and regeneration of skeletal muscle after injury or disease. MyoD family transcription factors play a key role to promote myoblast differentiation. In a screen for MyoD activators, we identified tetrahydropalmatine (THP), a natural compound isolated from *Corydalis turtschaninovii*. The treatment of C2C12 myoblasts with THP enhanced the level of MyoD, Myogenin and myosin heavy chain (MHC) proteins and the formation of larger multinucleated myotubes, compared to the control treatment. The THP treatment dramatically enhanced the activities of p38MAPK and Akt, the key promyogenic kinases which activate MyoD. The enhanced myoblast differentiation by THP treatment can be blocked by inhibition of p38MAPK or Akt by SB203580 or LY294002, respectively. In addition, THP treatment restored myotube formation of Cdo-depleted C2C12 cells through activation of p38MAPK. Moreover, THP enhanced the efficiency of trans-differentiation of 10T1/2 fibroblasts into myoblasts mediated by MyoD. These results indicate that THP has a promyogenic effect by upregulation of p38MAPK and Akt resulting in enhanced MyoD activation. Our findings suggest that THP has a potential as a therapeutic candidate to prevent fibrosis and improve muscle regeneration and repair.



## 1. Introduction

Sarcopenia is defined as the age-related loss of muscle mass and subsequently causes a negative health effect such as impaired strength, insulin resistance, a reduction in metabolic rate, increase of body fat mass and functionally leading to a reduced quality of life [1–4]. The extent of muscle loss that occurs during illness has been identified as an important predictor of the duration of hospitalization and subsequent need for rehabilitation [4]. Until now, the exact causes of sarcopenia and the mechanisms responsible for the loss of myofibers with aging remain unclear. The occurrence of sarcopenia or muscle atrophy seems to be attributed to decreased regenerative capacity of skeletal muscle stem cells. Therefore understanding the mechanisms of proliferation and differentiation of muscle stem cells during muscle regeneration is essential to develop therapeutic tools for sarcopenia and other muscle degenerative diseases.

Differentiation of skeletal myoblasts is a well-coordinated process involving myoblast proliferation, cell cycle withdrawal, expression of muscle-specific genes, and fusion into multinucleated myotubes [5]. The processes of myogenic specification and differentiation are coordinated by the myogenic transcriptional factors of the MyoD family [6]. The activity of MyoD occurs in conjunction with non-muscle-specific factors, including E proteins, Mef2 family, and transcriptional coactivators and repressors. The activity of MyoD is tightly regulated at the posttranslational level by promyogenic signaling pathways including p38MAPK and Akt [7–9]. p38MAPK regulates positively myogenesis at several levels, including cell cycle withdrawal, activation of MyoD by enhancing heterodimerization with its partner E protein, activation of Mef2 by phosphorylation, and changes in chromatin-remodeling at muscle-specific genes to facilitate gene transcription [8,10,11]. In addition, the Akt signaling pathway also plays an important role in myoblast differentiation [7]. The activation of Akt promotes myoblast differentiation, whereas the inhibition of Akt blocks

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myotube formation. Akt is a key regulator for cell survival which is essential for myoblast differentiation [12]. Both the p38MAPK and Akt pathways activate and reinforce each other's activity to induce efficient myogenic differentiation [13].

Moreover, cell-to-cell contact between muscle precursors promotes myoblast differentiation and a promyogenic receptor Cdo has been shown to be a critical component that integrates cell contact-mediated signals from the cell surface into the myogenic regulatory network [14]. Overexpression of Cdo in such cells enhances differentiation and the promyogenic activity of Cdo is largely exerted through the activation of the p38MAPK pathway and Cdo-deficient myoblasts exhibit defective myoblast differentiation accompanied by decreased p38MAPK activation [9,15].

The *Corydalis* tubers are used in traditional Korean medicine for a treatment of cardiac arrhythmia, rheumatism, and memory dysfunction, as well as a treatment for gastric ulcers. Several pharmacologically important alkaloids, such as corydaline, berberine, corptisine, and protopine are extracted from these plants [16–18]. In this study, we screened natural compounds purified from *Corydalis* tubers for MyoD-responsive reporter activities and myosin heavy chain expression (MHC) in myoblasts. Among others, we isolated tetrahydropalmatine (THP), a protoberberine-type alkaloid, from the tuber of *Corydalis turtschaninovii* (Papaveraceae). Although THP has been previously studied for its analgesic, sedative hypnotic, anxiolytic, and antihypertensive effects [16,19], its promyogenic effect has not been described yet. Therefore, we conducted experiments to examine the effect of THP on myoblast differentiation and the trans-differentiation of fibroblasts into myoblasts. The treatment with THP upregulated activation of two promyogenic kinases, p38MPAK and Akt led to MyoD activation and enhanced myoblast differentiation in C2C12 cells. In addition, THP treatment restored the defective differentiation caused by Cdo depletion via activation of p38MAPK. This is the first report that THP, a natural product, enhanced myoblast differentiation through activation of the promyogenic signaling pathways.

## 2. Materials and methods

### 2.1. Reagents

Fetal bovine serum (FBS) and Dulbecco modified Eagle's medium (DMEM) were purchased from Thermo Scientific (Waltham, MA). Horse serum (HS) was obtained from WelGene (Daegu, Korea). Lipofectamine 2000 was obtained from Invitrogen (Carlsbad, CA). SB203580 and LY294002 were obtained from CalBiochem (La Jolla, CA). Antibodies against phospho-p38MAPK, phospho-Akt, and Akt were purchased from Cell Signaling Technology (Beverly, MA). Myogenin and MyoD were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Cdo was purchased from Zymed Laboratories Inc. (South San Francisco, CA). MHC (MF-20) was obtained from the Developmental Studies Hybridoma Bank (Iowa, IA). Primary antibodies against pan-Cadherin and p38MAPK and all other chemicals used were obtained from Sigma–Aldrich (St. Louis, MO) unless stated otherwise.

### 2.2. General experimental procedures

The organic solvents for solid extraction and solvent fractionation were first-grade reagents supplied by Daejung Chemical Ltd. (Seoul, Korea). $SiO_2$ resin used for column chromatography was Kiesel gel 60 (Merck, Darmstadt, Germany). TLC analysis was carried out using Kiesel gel 60 $F_{254}$ and RP-18 $F_{254S}$ (Merck) and detected using a UV lamp, Spectroline Model ENF-240 C/F (Spectronics Corporation, New York, NY) and a 10% $H_2SO_4$ solution. The melting point was determined on a Fisher-John's apparatus and was not corrected. Optical rotation was measured on a JASCO P-1010 digital polarimeter (Tokyo, Japan). IR spectrum was obtained from a Perkin Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). EI-MS was recorded on a JEOL JMSAX-700 (Tokyo, Japan). $^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectra were recorded on a Varian Unity Inova AS-400 FT-NMR spectrometer (Palo Alto, CA).

### 2.3. Extraction and isolation from the tuber of C. turtschaninovii

The tubers of *C. turtschaninovii* were purchased at Kyungdong Herb Medicine Market in Seoul in 2012 and were identified by Professor Dae-Keun Kim (Woosuk University, Jeonju, Korea). A voucher specimen (KHU070123) was reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea. The dried and coarsely powdered plant materials (2 kg) were extracted with 80% aqueous MeOH (3 L × 2) at room temperature overnight. The filtrate of extracts was evaporated *in vacuo* giving a dark brownish residue. The resultant methanolic extract was poured into acidic water (pH 2.5, 1.1 L), the acidity of which was adjusted using 30% HCl, and washed twice with EtOAc (1.1 L × 2). The aqueous layer was alkalized to pH 11.5 using a 20% NaOH solution and extracted with EtOAc (1.1 L × 2). The concentrated organic layer (CTE, 6.2 g) was subjected to column chromatography (6 × 8 cm) over $SiO_2$ (70–230 mesh, 200 g) using $CHCl_3$–MeOH (15:1 → 13:1 → 10:1 → 7:1 → 5:1 → 3:1, each 1.2 L) and MeOH (0.8 L) as eluting solvents in increasing order of polarity to give 13 fractions (CTE1–CTE13). The fourth fraction (CTE4, 2500–4200 mL, 770 mg) was applied to $SiO_2$ column chromatography (70 g, 3 × 6 cm) using *n*-hexane-EtOAc (1:5, 2 L) as the eluent to yield six sub-fractions (CTE4-1–CTE4-6) including a pure compound 1 (760–1000 mL, 170 mg, $R_f$ 0.39, Kiesel gel 60 $F_{254}$, *n*-hexane-EtOAc = 3:1].

### 2.4. Cell cultures

Myoblast C2C12 cells and embryonic fibroblast 10T1/2 cells were cultured as described previously [7,20]. To induce differentiation of C2C12 myoblasts, cells at near confluence were switched from DMEM containing 15% FBS (growth medium, GM) to DMEM containing 2% HS (differentiation medium, DM) and myotube formation was observed normally at approximately 2–3 days of differentiation. The efficiency of the myotube formation was quantified by a transient differentiation assay as previously described [7]. To generate C2C12 cells that transiently overexpress shRNA against Cdo, cells were transfected with the indicated expression vector and Lipofectamine 2000, and cultures were selected in puromycin-containing medium. For our experiments that involved p38MAPK and Akt inhibitors, C2C12 cells were treated with 500 nM THP after pre-incubation with SB203580 and LY294002 in fresh culture medium for 30 min. For the trans-differentiation study, 10T1/2 cells were cultured in DMEM supplemented with 10% FBS in 10-cm plates, and transfected with 5 μg of MyoD or control vector. After 1–2 day incubation, cells were treated with 500 nM THP in 2% HS for 2 days.

### 2.5. Western blotting analysis

Western blot analysis was performed as previously described [7]. The cells were lysed in an extraction buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 10% glycerol, 1.5 mM $MgCl_2$, 1 mM EGTA, 1% Triton X-100, 10 mM NaF, 1 mM $Na_3VO_4$, Complete protease inhibitor cocktail) and sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis was performed. The primary antibodies used were anti-Cdo, anti-pan-Cadherin, anti-p38, anti-p-p38 (the

phosphorylated, active form of p38MAPK), Akt, p-Akt (the phosphorylated, active form), anti-MHC, anti-Myogenin, and MyoD.

### 2.6. Immunocytochemistry and microscopy

Immunostaining for MHC expression was performed as described previously [20]. Briefly, C2C12 cells were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 in phosphate buffered saline, blocked, and stained with anti-MHC, followed by an Alexa Fluor 568-conjugatd secondary antibody (Molecular Probes). Images were captured and processed with a Nikon ECLIPSE TE-2000U microscope and NIS-Elements F software (Nikon) and Photoshop CS5 (Adobe).

### 2.7. Statistics

The experiments were done independently three times. The participants' *T*-test was used to access the significance of the difference between two mean values. $^{*}P < 0.01$ and $^{**}P < 0.05$ were considered to be statistically significant.

## 3. Results and discussion

### 3.1. THP promotes myoblast differentiation

To identify a potential activator of MyoD and myoblast differentiation, we have generated the MyoD-responsive reporter 4RTK expressing C2C12 mouse myoblasts and these cells were used to screen with natural compound purified from *Corydalis* tubers. To evaluate the effect from the first round of the MyoD-reporter screening, the selected compounds were assessed for their induction ability of MHC expression in C2C12 cells. Among the candidate compounds, THP purified from *C. turtschaninovii* showed consistent results from both screening experiments (Fig. 1A). Therefore we further assessed the mechanism of THP in activation of MyoD and myoblast differentiation. To examine the effect of THP on myogenic differentiation, C2C12 myoblasts were induced to differentiate for 2 days (D2) in the presence of various concentrations of THP (100–1000 nM). We did not observe any overt cytotoxic effect of THP treatment in C2C12 myoblasts under both growth and differentiation conditions, as assessed by Annexin-V/PI staining (data not shown). The treatment of C2C12 cells with THP enhanced the expression muscle-specific proteins, including MHC, MyoD, and Myogenin in a dose dependent manner (Fig. 1B). In addition, the promyogenic cell surface protein Cdo was also transiently up-regulated in THP-treated C2C12 cells (Fig. 1B). In addition, the vehicle- and THP-treated C2C12 cells were induced to differentiate for 2 days followed by immunostaining with anti-MHC antibodies and DAPI staining to assess myotube formation. The treatment of C2C12 cells with THP resulted in the formation of larger myotubes with more nuclei per myotube, in comparison to vehicle-treated cells (Fig. 1C and D). These data suggest that THP enhanced myoblast differentiation of C2C12 cells at a morphological as well as a biochemical level.

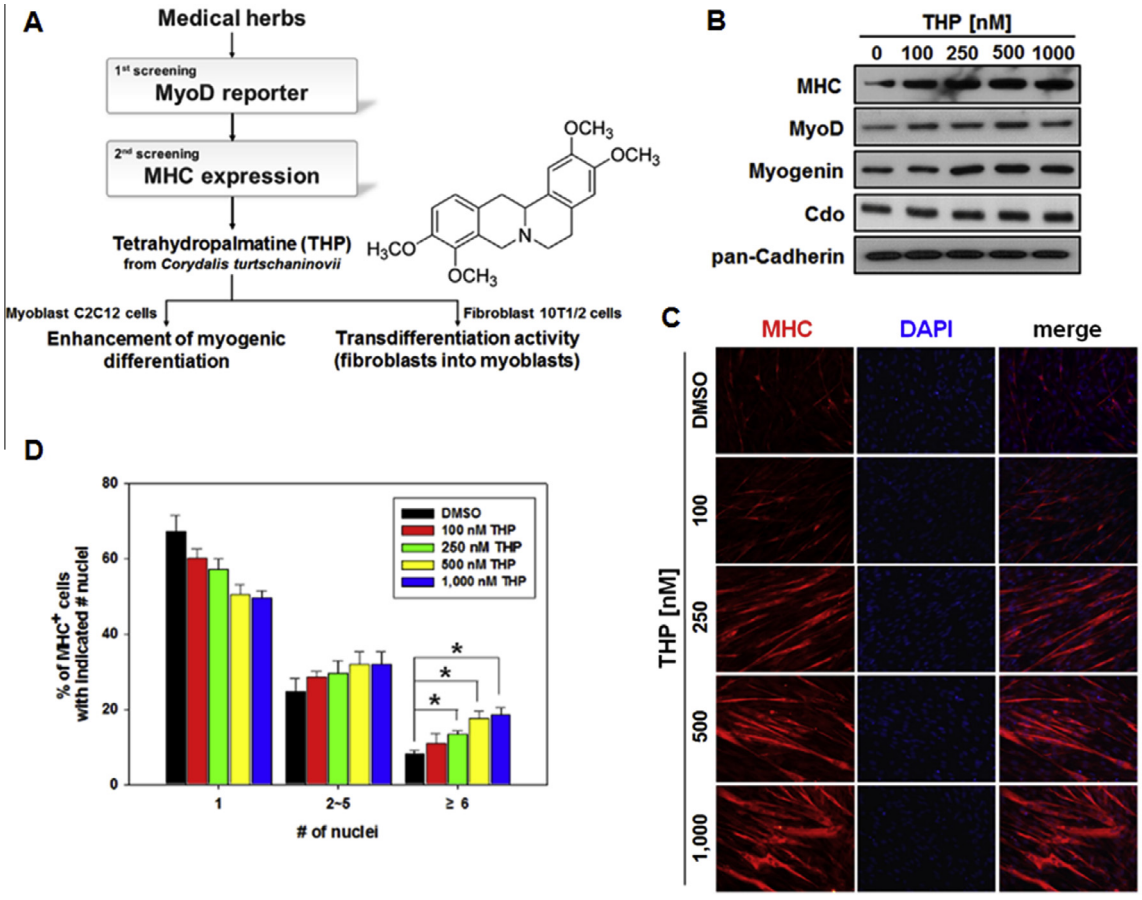


**Fig. 1.** THP promotes myogenic differentiation of C2C12 cells. (A) Schematic diagram and chemical structure of THP. (B) C2C12 cells were treated with THP and differentiated in DM for 2 days. Cell lysates were Western blotted with antibodies to Cdo, MHC, MyoD, Myogenin, and pan-Cadherin. The experiment was repeated three times with similar results. (C) Cells from B were stained for MHC expression (red) and with DAPI to stain nuclei (blue) to reveal myotube formation. (D) Quantification of myotube formation in the cell lines shown in panel C. Values represent means of triplicate determinations ±1 standard deviation (SD). The experiment was repeated three times with similar results. Asterisks indicate significant difference from the control at $^{*}P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

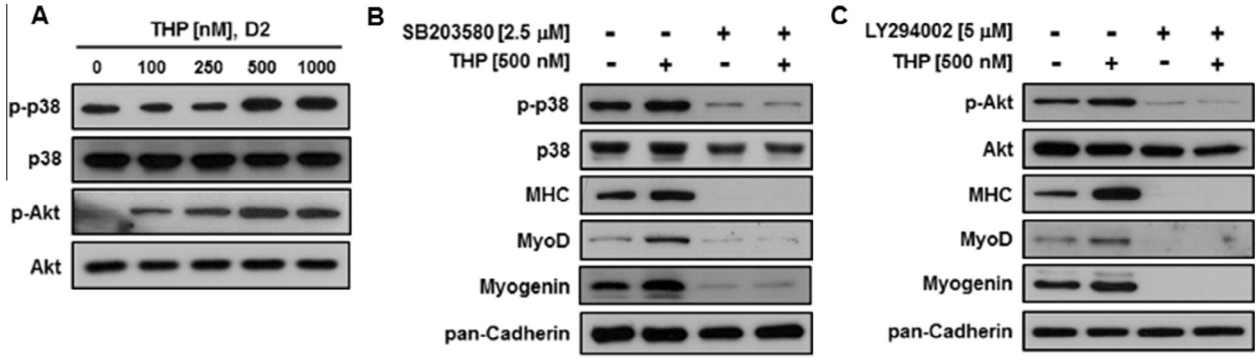


**Fig. 2.** THP induces the phosphorylation of p38MAPK and Akt dose-dependently. (A) C2C12 cells were treated with THP and differentiated in DM for 2 days. Cell lysates were Western blotted with antibodies to p-p38MAPK (p-p38), p38MAPK (p38), p-Akt, and Akt. The experiment was repeated three times with similar results. (B and C) C2C12 cells were treated with SB203580 (B) or LY294002 (C), respectively, for 30 min before treatment with THP, and then differentiated in DM for 2 days. Cell lysates were Western blotted with antibodies to p-p38MAPK, p38MAPK, p-Akt, Akt, MHC, MyoD, Myogenin and pan-Cadherin as a loading control.

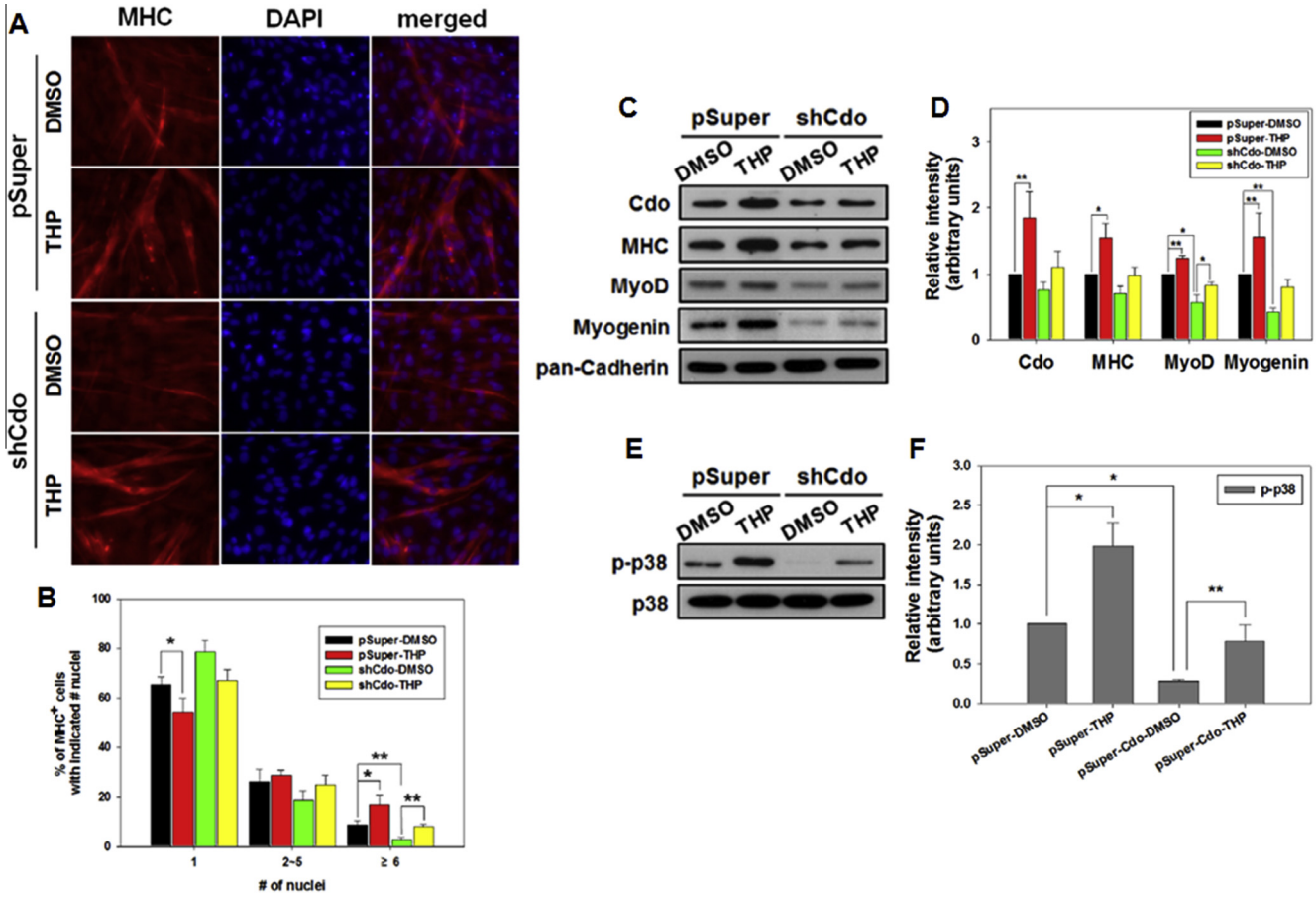


**Fig. 3.** THP rescues myogenesis in Cdo-depleted C2C12 cells. (A) C2C12/pSuper and C2C12/Cdo shRNA cells were transiently transfected with control or Cdo shRNA, and induced to differentiate with treatment of either 500 nM THP or DMSO for 2 days, followed by immunostaining with antibodies against MHC, and DAPI staining. (B) Quantification of myotube formation shown in panel A. Values represent means of triplicate determinations ±1 SD. The experiment was repeated three times with similar results. Significant difference from control, $^{*}P < 0.01$ and $^{**}P < 0.05$. (C) Lysates of cell lines shown in panel A were Western blotted with antibodies to Cdo, MHC, MyoD, Myogenin, and pan-Cadherin as a loading control. (D) Quantification of the three independent experiments, one of which was shown in panel C. The intensity of myogenic-specific proteins was quantified, and the values from pSuper-DMSO were set to 1.0. Values represent the means of triplicate determinations ±1 SD. The experiment was repeated three times with similar results. Significant difference from control, $^{*}P < 0.01$ and $^{**}P < 0.05$. (E) Lysates of cell lines shown in panel A were Western blotted with antibodies to p-p38MAPK and p38MAPK. (F) Quantification of the three replicate blots, which was shown in panel E. The intensity of the p-p38MAPK was quantified, and the values obtained from p38MAPK respectively, were set to 1.0. Values represent the means of triplicate determinations ±1 SD. Significant difference from vehicle-treated group, $^{*}P < 0.01$ and $^{**}P < 0.05$.

### *3.2. THP activates p38MAPK and Akt pathways in myoblast differentiation*

To examine the effect of THP treatment on promyogenic signaling pathways, we have treated C2C12 cells with various amount of THP and assessed the activation levels of p38MAPK and Akt by Western blot analysis using antibodies recognizing an active, phosphorylated form of these kinases. As shown in Fig. 2A, THP treatment significantly increased the levels of phosphorylated-p38MAPK (p-p38) and phosphorylated-Akt (p-Akt) in

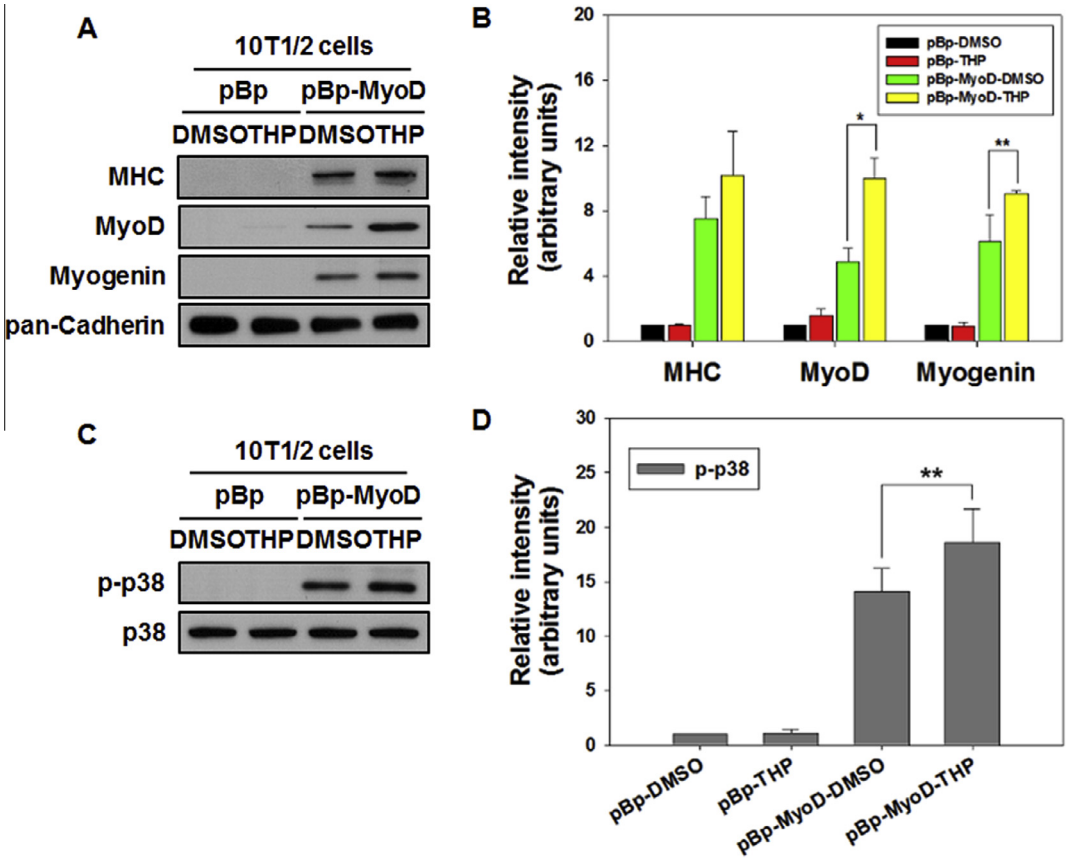


**Fig. 4.** THP trans-differentiates MyoD-transfected 10T1/2 fibroblasts into myoblasts. 10T1/2 cells were transiently transfected with control (pBp) or MyoD expression vector (pBp–MyoD) and induced to differentiate with treatment of either 500 nM THP or DMSO for 2 days. Cell lysates were Western blotted with antibodies to MHC, MyoD, Myogenin, pan-Cadherin (A), and p-p38MAPK, p38MAPK (C). The experiment was repeated three times with similar results. (B) Quantification of the three independent experiments, one of which was shown in panel A. The intensity of myogenic-specific proteins was quantified, and the values from pBp–DMSO were set to 1.0. Values represent the means of triplicate determinations ±1 SD. The experiment was repeated three times with similar results. Significant difference from vehicle-treated group, $^{*}P < 0.01$ and $^{**}P < 0.05$. (D) Quantification of the three blots replicate immunoblots, one of which was shown in panel C. The intensity of the p-p38MAPK was quantified, and the values obtained from p38MAPK respectively, were set to 1.0. Values represent the means of triplicate determinations ±1 SD. Significant difference from vehicle-treated group, $^{*}P < 0.01$ and $^{**}P < 0.05$.

a dose-dependent manner while the total protein levels stayed relatively constant. We next assessed whether THP treatment can override the block of myoblast differentiation by inhibition of p38MAPK or Akt. To do so, C2C12 cells were treated with pharmacological inhibitors SB203580 or LY294002 for 30 min prior to the addition of THP and then differentiated for 2 days, followed by Western blot analysis. As predicted, the treatment of SB203580 or LY294002 reduced the phosphorylation of p38MAPK or Akt, respectively, and inhibited the expression of muscle-specific proteins, MHC, MyoD and Myogenin (Fig. 2B and C). Furthermore THP treatment did not restore the activation status of p38MAPK or Akt and the expression of MHC, MyoD and Myogenin under the condition of pretreatment with SB203580 or LY294002. In addition, SB203580 pretreatment also reduced Akt phosphorylation, and the THP slightly rescued Akt phosphorylation in C2C12 cells co-treated with this inhibitor (data not shown). It has been reported that a reciprocal cross-talk and activation between p38MAPK and Akt pathways is essential for efficient myoblast differentiation. Complementary to p38MAPK-mediated transactivation of Akt, activation or inhibition of PI3K regulates p38MAPK activity, upstream of MKK6, resulting in a reciprocal communication to reinforce each other's activities forming a positive feedback loop characteristic of myogenic regulation [13]. These results indicate that THP accelerates myoblast differentiation through the activation of p38MAPK and Akt signaling and its promyogenic effect is dependent on p38MAPK and Akt activation.

### 3.3. THP rescues myogenic differentiation in Cdo-depleted C2C12 cells

A promyogenic receptor protein Cdo regulates myoblast differentiation mainly through p38MAPK activation [9,15]. Our data suggested that THP treatment promotes myoblast differentiation via activation of p38MAPK. We investigated whether defective myoblast differentiation caused by Cdo depletion can be rescued by treatment with THP. To test this, C2C12 cells were transiently transfected with pSuper and Cdo shRNA expression vectors. C2C12/pSuper and C2C12/Cdo shRNA cells were then treated with a vehicle or THP and induced to differentiate for 2 days, followed by immunostaining with anti-MHC antibodies and DAPI staining. Consistently the THP-treatment of C2C12/pSuper cells enhanced myotube formation with a higher proportion of large myotubes containing more than six nuclei compared with the vehicle-treated cells. In agreement with previously reported results, C2C12/Cdo shRNA cells treated with vehicle displayed impaired myotube formation which was restored by the THP treatment almost to the level of the control cells (Fig. 3A and B). Western blot analysis of these cells at D2 revealed that the depletion of Cdo is relatively modest in C2C12/Cdo shRNA cells however the expression of muscle specific proteins MHC, MyoD and Myogenin was substantially decreased in these cells. In addition, the treatment of THP in C2C12/Cdo shRNA cells restored partially the expression of muscle specific proteins MHC and MyoD, compared to the control C2C12/pSuper cells (Fig. 3C and D). Interestingly, THP treatment in C2C12/

Cdo shRNA restored the expression of Cdo to a similar level of control cells. This may contribute to the restored myoblast differentiation. It has been previously reported that Cdo forms a positive feedback loop with MyoD in myoblast differentiation [15], therefore the reexpression of Cdo by THP treatment may be through MyoD activation. To assess whether THP treatment can restore p38MAPK activation, p-p38 levels were assessed by Western blot analysis. The level of p-p38 was also significantly decreased in C2C12/Cdo shRNA cells which was then restored by THP treatment almost to level of C2C12/pSuper cells treated with vehicle. Taken together, the data presented here indicate that THP restores p38MAPK activation resulting in activation of MyoD and Cdo expression thereby promoting MHC expression and myotube formation.

### *3.4. THP enhances the trans-differentiation potency of embryonic fibroblasts into myoblasts*

Using 10T1/2 fetal embryonic fibroblasts as a model system, we examined whether THP treatment can enhance MyoD-mediated trans-differentiation of 10T1/2 fibroblasts into myoblasts. 10T1/2 cells were transfected with pBp (pBabe-puro) and pBp/MyoD expression vectors, then treated with DMSO or THP and induced to differentiate for 2 days, followed by Western blot analysis. As shown in Fig. 4A and B, no expression of muscle-specific proteins observed in pBp-transfected 10T1/2 cells, irrespective of THP treatment. On the other hand, MyoD, Myogenin, and MHC were expressed in pBp/MyoD-transfected 10T1/2 cells. When treated with THP in pBp/MyoD-transfected 10T1/2 cells, the level of MyoD expression was dramatically increased relative to that of the vehicle-treated cells, and Myogenin and MHC expressions were substantially increased in these cells. Next we have tested whether p38MAPK activation is involved in this activity. The transdifferentiation of 10T1/2 cells by MyoD transfection enhanced the level of p-p38 and the treatment of THP in pBp/MyoD-transfected 10T1/2 cells further increased p-p38 levels (Fig. 4C and D). These results further support that THP can enhance the activity of MyoD via p38MAPK activation thereby promoting myoblast differentiation as well as enhancing the trans-differentiation of fibroblasts into myoblasts mediated MyoD.

Taken together, THP isolated from *C. turtschaninovii* promotes myoblast differentiation through enhancing the activation of promyogenic signaling network from p38MAPK and/or Akt to MyoD. Our findings firstly provide new insight into molecular mechanisms by which THP promotes myoblast differentiation and suggests a mechanistic rationale for the pharmaceutical application of THP in an age-related sarcopenia or skeletal muscle atrophy.

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## References

[1] N. Brooks, G.J. Cloutier, S.M. Cadena, J.E. Layne, C.A. Nelsen, A.M. Freed, R. Roubenoff, C. Castaneda-Sceppa, Resistance training and timed essential amino acids protect against the loss of muscle mass and strength during 28 days of bed rest and energy deficit, J. Appl. Physiol. 105 (2008) (1985) 241–248.
[2] D.G. Candow, Sarcopenia: current theories and the potential beneficial effect of creatine application strategies, Biogerontology 12 (2011) 273–281.
[3] H. Karakelides, K.S. Nair, Sarcopenia of aging and its metabolic impact, Curr. Top. Dev. Biol. 68 (2005) 123–148.
[4] B.T. Wall, M.L. Dirks, L.J. van Loon, Skeletal muscle atrophy during short-term disuse: implications for age-related sarcopenia, Ageing Res. Rev. 12 (2013) 898–906.
[5] V. Horsley, G.K. Pavlath, Forming a multinucleated cell: molecules that regulate myoblast fusion, Cells Tissues Organs 176 (2004) 67–78.
[6] M.E. Pownall, M.K. Gustafsson, C.P. Emerson Jr., Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos, Annu. Rev. Cell Dev. Biol. 18 (2002) 747–783.
[7] G.U. Bae, J.R. Lee, B.G. Kim, J.W. Han, Y.E. Leem, H.J. Lee, S.M. Ho, M.J. Hahn, J.S. Kang, Cdo interacts with APPL1 and activates Akt in myoblast differentiation, Mol. Biol. Cell 21 (2010) 2399–2411.
[8] L. de Angelis, J. Zhao, J.J. Andreucci, E.N. Olson, G. Cossu, J.C. McDermott, Regulation of vertebrate myotome development by the p38 MAP kinase–MEF2 signaling pathway, Dev. Biol. 283 (2005) 171–179.
[9] G. Takaesu, J.S. Kang, G.U. Bae, M.J. Yi, C.M. Lee, E.P. Reddy, R.S. Krauss, Activation of p38alpha/beta MAPK in myogenesis via binding of the scaffold protein JLP to the cell surface protein Cdo, J. Cell Biol. 175 (2006) 383–388.
[10] C. Serra, D. Palacios, C. Mozzetta, S.V. Forcales, I. Morantte, M. Ripani, D.R. Jones, K. Du, U.S. Jhala, C. Simone, P.L. Puri, Functional interdependence at the chromatin level between the MKK6/p38 and IGF1/PI3K/AKT pathways during muscle differentiation, Mol. Cell 28 (2007) 200–213.
[11] C. Simone, S.V. Forcales, D.A. Hill, A.N. Imbalzano, L. Latella, P.L. Puri, P38 pathway targets SWI–SNF chromatin-remodeling complex to muscle-specific loci, Nat. Genet. 36 (2004) 738–743.
[12] M.A. Lawlor, P. Rotwein, Insulin-like growth factor-mediated muscle cell survival: central roles for Akt and cyclin-dependent kinase inhibitor p21, Mol. Cell. Biol. 20 (2000) 8983–8995.
[13] I. Gonzalez, G. Tripathi, E.J. Carter, L.J. Cobb, D.A. Salih, F.A. Lovett, C. Holding, J.M. Pell, Akt2, a novel functional link between p38 mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways in myogenesis, Mol. Cell. Biol. 24 (2004) 3607–3622.
[14] R.S. Krauss, F. Cole, U. Gaio, G. Takaesu, W. Zhang, J.S. Kang, Close encounters: regulation of vertebrate skeletal myogenesis by cell–cell contact, J. Cell Sci. 118 (2005) 2355–2362.
[15] J.S. Kang, G.U. Bae, M.J. Yi, Y.J. Yang, J.E. Oh, G. Takaesu, Y.T. Zhou, B.C. Low, R.S. Krauss, A Cdo–Bnip-2–Cdc42 signaling pathway regulates p38alpha/beta MAPK activity and myogenic differentiation, J. Cell Biol. 182 (2008) 497–507.
[16] M.T. Lin, F.Y. Chueh, M.T. Hsieh, C.F. Chen, Antihypertensive effects of DL-tetrahydropalmatine: an active principle isolated from *Corydalis*, Clin. Exp. Pharmacol. Physiol. 23 (1996) 738–742.
[17] H. Matsuda, H. Shiomoto, S. Naruto, K. Namba, M. Kubo, Anti-thrombic action of methanol extract and alkaloidal components from *Corydalis* tuber, Planta Med. 54 (1988) 27–33.
[18] K.J. Yun, J.S. Shin, J.H. Choi, N.I. Back, H.G. Chung, K.T. Lee, Quaternary alkaloid, pseudocoptisine isolated from tubers of *Corydalis turtschaninovii* inhibits LPS-induced nitric oxide, PGE(2), and pro-inflammatory cytokines production via the down-regulation of NF-kappaB in RAW 264.7 murine macrophage cells, Int. Immunopharmacol. 9 (2009) 1323–1331.
[19] W.C. Leung, H. Zheng, M. Huen, S.L. Law, H. Xue, Anxiolytic-like action of orally administered DL-tetrahydropalmatine in elevated plus-maze, Prog. Neuropsychopharmacol. Biol. Psychiatry 27 (2003) 775–779.
[20] G.U. Bae, B.G. Kim, H.J. Lee, J.E. Oh, S.J. Lee, W. Zhang, R.S. Krauss, J.S. Kang, Cdo binds Abl to promote p38alpha/beta mitogen-activated protein kinase activity and myogenic differentiation, Mol. Cell. Biol. 29 (2009) 4130–4143.