Targeting of the Sonic Hedgehog Pathway by Atractylenolides Promotes Chondrogenic Differentiation of Mesenchymal Stem Cells

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Molecules that enhance chondrogenic differentiation in mesenchymal stem cells (MSCs) were identified and isolated using an in vitro Gli reporter gene assay in MSCs incorporating a Sonic Hedgehog (Shh) target. Atractylenolide III, which promoted Gli1-mediated transcriptional activity, was isolated from an ethyl acetate extract of the Rhizoma, Atractylodis macrocephalae. After dehydration, atractylenolide III was transformed to atractylenolide I. Both atractylenolides were confirmed by MS, UV, IR, 1H- and 13C-NMR spectra. Atractylenolide III (which contains –OH at the 8-position) and atractylenolide I (which lacks –OH at the 8-position) were found to effectively promote the activity of the Gli promoter. While the hydroxyl group of atractylenolide III was not essential for the effect of atractylenolide, its effect was dependent on Shh signaling. Phenotypic cellular analysis indicated that atractylenolides induced MSCs to differentiate into chondrocytes, as shown by increased expression of specific chondrogenic markers including collagen II, aggrecan and the cartilage related transcription factor, Sox9. Atractylenolides significantly increased the expression of Shh and its target gene Gli-1, indicating that Shh signaling was activated by atractylenolides. Moreover, inhibition of Shh signaling reduced the effect of atractylenolides on the chondrogenic phenotype. The discovery that atractylenolides induce chondrocytes from MSCs is promising for bony disease therapy.

Key words atractylenolide; chondrogenic differentiation; mesenchymal stem cell; sonic hedgehog signaling

Mesenchymal stem cells (MSCs) in adult bone marrow are capable of self-renewal and can differentiate into all mesodermal cell types and neuro-ectodermal cell types such as osteoblasts, chondrocytes, myoblasts, stromal cells, adipocytes, neurons and astrocytes.5) Under appropriate culture conditions, MSCs can differentiate into chondrocytes.2) Recent studies showed that implanted MSCs can differentiate into chondrocytes within cartilage defects in animal models.3) However, direct implantation of undifferentiated MSCs caused the calcification of implanted cells, fibrogenesis, and heterotopic tissue formation in cartilage.4) Therefore, chondrogenic differentiation is crucial to the efficacy of MSC-based cartilage regeneration. Previous studies reported that various signaling pathways or transcription factors contributed to the initiation of the chondrogenic process.5) Among them, Sonic Hedgehog (Shh), a secreted glycoprotein expressed in the notochord and floor plate, plays a critical role in the control of chondrogenic differentiation.6) Shh signaling is initiated by the binding of the Shh ligand to its transmembrane receptor, patched, which relieves the suppression of the transmembrane protein, smoothed (smo). Smo activates an intracellular cascade that results in the activation of Gli transcription factors.7) Shh promotes chondrogenesis in mesenchymal cells by inducing the expression of Sox9.8,9) Shh and Gli genes play an essential role in cartilage development,9,10) and thus it has been proposed that Shh is an important pharmacological target for signaling pathways that mediate chondrogenic differentiation in MSCs. Therefore, Shh can be used effectively in screens for new drugs from medicinal plants, particularly traditional Chinese herbs.

The rhizoma, Atractylodis macrocephalae, is a traditional Chinese herb widely used as a tonic agent and for treatment of arthritis, which contains several sesquiterpenes including atractylenolides I and III. It was reported that atractylenolide III possesses various pharmacological activities, such as anti-inflammatory activity,11-13) anti-cancer activity,14,15) protective effects,16) and inhibition of the movement of uterus smooth muscle.17) In this study, an in vitro Gli reporter gene assay in MSCs was used to isolate Shh signaling activators, in order to identify molecules that enhance chondrogenic differentiation in MSCs.

MATERIALS AND METHODS

Plants, Animals and Materials The rhizoma, Atractylodis macrocephalae, was purchased from the Yanghe Pharmacy at Guangzhou University of Chinese Medicine (Guangzhou, China), and authenticated by Professor Shuhui Tan. Embryonic day 14 Sprague-Dawley rats were obtained from the animal center at Guangzhou University of Chinese Medicine. All animals received human care in accordance with the guidelines set by the Care of Experimental Animals Committee of Guangzhou University of Chinese Medicine. Chromatography columns were filled with silica gel GF254 (Qindao Haiyang Chemical Co., Ltd., China). Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen Co., Ltd., U.S.A. Shh and Sox9 antibody were provided by Santa Cruz Biotechnologies, U.S.A. Collagen II, aggrecan and actin antibodies were purchased from Wuhan Boster Biological Technology Co., Ltd., China. Shh was purchased from R&D Systems (Minneapolis, U.S.A.). Horseradish-peroxidase was purchased from New England Biolabs. Reverse transcription-polymerase chain reaction (RT-PCR) reagent, for the synthesis of cDNA, was obtained from QIAGEN, Germany. The chemiluminescence

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kit was purchased from Invitrogen, U.S.A. Cyclopamine and dimethyl sulfoxide (DMSO) were obtained from Sigma (Sigma Aldrich Trading Co., Shanghai, China). PGL3-Basic Vector and the pCMV plasmid were kindly provided by Dr. Huang Qilai and Dr. Chen Yuan (State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, China). Methanol and water were of High Performance Liquid Chromatography (HPLC) grade and other chemicals were of analytical grade.

**Preparation of Atractylenolides I and III** The rhizoma, *Atractylodis macrocephalae*, was soaked in ethyl acetate at room temperature for 10 d, following which the extract was collected. This procedure was performed three times in total, and the extracts were pooled and filtered through filter paper. Following concentration with a rotary evaporator, the filtrate was applied onto a silica gel chromatography column and eluted with petroleum ether–absolute ethanol (100 : 0) and then with petroleum ether–absolute ethanol (90 : 1), and finally with petroleum ether–absolute ethanol (80 : 2). Crude Atractylenolide was dehydrated and yielded atractylenolide I (99% purity, Chart 1).

**Identification of Atractylenolides I and III** The 1H- and 13C-NMR spectra were recorded in CDCl3 with a Bruker 500 MHz NMR spectrometer (Bruker Biospin, Billerica, MA, U.S.A.). Electron ionization (EI)-MS was recorded on a DSQ mass spectrometer (Thermo, U.S.A.). The IR spectrum was determined using a Fourier transform infrared spectrophotometer (Thermo, U.S.A.). Electron ionization (EI)-MS was recorded on a Techcomp LC2000 system equipped with a UV detector (Techcomp Co., Ltd., Shanghai, China). HPLC analysis was performed using a Techcomp UV-2100 spectrophotometer (Shanghai, China). The chromatographic conditions were: Dikma Diamonsil C18 (250 mm × 4.6 mm, 5 μm); mobile phase, methanol–0.1% formic acid (85 : 15); flow rate, 0.5 mL/min; injection volume, 2 μL (2 mg/mL); detection wavelength, 280 nm. Melting points were determined on WRS-1B digital melting point apparatus (Shengguang, Shanghai, China) and were corrected by benzoic acid.

Atractylenolide I: A white needle crystal of 99% purity as determined by HPLC analysis, C15H18O2, MW 230, mp 109–111°C. MS m/z: 231 [M+H]+, 203, 185; UV λmax = CH2 (1665.40, 895.93 cm−1); −CH2− (2935.64 cm−1); C=O (1770.28 cm−1); C=C (1650.10 cm−1); −CH3 (1378.57 cm−1); C=O–CO (1233.12 cm−1). 1H-NMR (CDCl3) δ: 0.929 (s, 3H), 1.601–1.699 (m, 4H), 1.896–1.891 (s, 3H), 2.036–2.045 (m, 1H), 2.317–2.381 (m, 2H), 2.487–2.551 (m, 1H), 2.661–2.702 (m, 1H), 4.619 (s, 1H), 4.902 (s, 1H), 5.602 (s, 1H). 13C-NMR (CDCl3) δ: 8.497, 18.584, 22.667, 23.001, 36.180, 38.131, 39.079, 48.394, 119.182, 120.446, 147.988, 148.070, 149.420, 152.640, 161.240, 172.730 (Supplemental Information Fig. S8–S12).

**Plasmids, Cell Transfection and Luciferase Assays** To construct the Gli promoter-Luc vector, 2.3 kb (~2303/+) of the 5′-flanking region of the rat Gli gene was amplified by PCR using the forward primer, 5′-CTGCTCTGAGAGTTTGTGATCCCGGTGTG-3′ and the reverse primer, 5′-CCCAAGCTTGGTCTCTACGAGAATGG-3′. The PCR product and pGL3-Basic vector were digested with *Nhel* and *HindIII* and ligated together to generate the Gli promoter-Luc vector. pRL-SV40 was cotransfected to normalize the variations in transfection efficiency.

MSCs were cultured in DMEM containing 10% FBS at 37°C in 5% CO2. MSCs were divided into different groups for determining the effects of atractylenolides, Shh or cyclopamine on Gli promoter activity. MSCs seeded in culture medium without treatment served as controls. Atractylenolides and cyclopamine were each dissolved in DMSO. An equal volume of DMSO was added to the control cultures. For dose–response analysis of the activity of atractylenolides on the Gli promoter, MSCs were stimulated with atractylenolide concentrations between 0–300 μg/mL or with 500 ng/mL of Shh. For co-treatment experiments, MSCs were stimulated with 250 ng/mL of Shh or cyclopamine or with 250 ng/mL of Shh. When luciferase reporter constructs were used, luciferase assays were performed with the Dual Luciferase Assay Kit (Promega) according to the manufacturer’s instructions. Cell lysates (10 μL) were first assayed for firefly luciferase and then for Renilla luciferase activity. Firefly luciferase activity was normalized to Renilla luciferase activity.

**Culture of MSCs** Bone marrow was obtained from the femur and tibia of rat. All experimental procedures were approved by the Care of Experimental Animals Committee.
of Guangzhou University of Chinese Medicine. The marrow samples were diluted with DMEM (low glucose, LG) containing 10% FBS. MSCs were prepared by gradient centrifugation at 900×g for 30 min on Percoll of a density of 1.073 g/mL. The cells were washed, counted and plated at 1×10⁶/cm² on Petri dishes in DMEM-LG supplemented with 10% FBS. Medium was replaced and the unattached cells were removed every 3 d. MSCs cultured as confluent flasks for 9 d were detached by treatment with 0.25% trypsin and passaged into culture flasks at 1×10⁶/cm². MSCs at passage 3 were evaluated for homogeneity in culture by CD 44 detection with flow cytometry. The cells used for the experiments were described as 95% homogeneous. MSCs were verified on the basis of their ability to differentiate into osteocytes, adipocytes and chondrocytes, as described by Pittenger et al.⁵

**Chondrogenic Induction** MSCs at passage 3 were plated in 24-well plates or 6-well plates at 8000 cells/cm². Twenty-four hours after cell plating, atractylenolides were added to induce cell differentiation. On day 7 of differentiation, Q-RT-PCR, immunostaining and Western blot analysis were performed for chondrogenic markers and the Shh signal pathway.

**Immunocytochemistry** Cells were immunostained with rat anti-collagen II or anti-aggrecan primary antibodies (1:200) overnight, followed by incubation with the secondary antibody. Diaminobenzidine (DAB) staining was employed to detect the expression of collagen II or aggrecan according to the manufacturer’s instructions. Subsequently, hematoxylin was used as a counter stain and the positive signal appeared as dark brown color within the cytoplasm. The control cells were stained using an identical procedure with non-immune serum replacing the primary antibody. The percentage of positive cells vs. total cells in each field was assessed.

**Real Time RT-PCR Analysis** MSCs were plated and then treated with atractylenolides for 7 d. Cells were harvested for purification of RNA. Total cellular RNA was extracted using Trizol reagent according to the manufacturer’s instructions. Total RNA (5 µL) was reverse-transcribed into cDNA and then amplified by fluorescent quantity PCR using the ABI prism 7900 HT Sequence Detection system. The fluorescent quantity PCR conditions were: pre-denaturation at 93°C for 2 min, then 40–45 cycles at 93°C for 45 s, and 55°C for 1 min. The relative mRNA expression levels were normalized to GAPDH. The primers were as follows: collagen II, sense, 5'-GAGCGG TCCAGTTCATGTG-3'; and antisense, 5'-GGCATGGTTGC TATGCACTCAAG-3'; aggrecan, sense, 5'-CCTGCTCACTG CCACTTTGA-3'; and antisense, 5'-GGGAGGCCAGGG CAAATCTA-3'; Sox9, sense, 5'-GGGACCTCGACAA GCCT-3'; and antisense, 5'-GCCACCGGGG-3'; Gli-1, sense, 5'-TCTTGA CCAAGGGGCTCTTC-3'; Shh, sense, 5'-AAAGCT GACGCTTTGA-3'; and antisense, 5'-GAGGGAT GTTATGCTC-3'; Gli promoter activity was also observed with Shh, which used the same primers for differentiation of MSCs into chondrocytes.

**RESULTS**

**Atractylenolides Increase Gli Promoter Activity** To investigate whether atractylenolides activate the Gli promoter, MSCs were transfected with a Gli promoter reporter construct and then either left unstimulated or stimulated with the indicated concentrations of atractylenolide I, atractylenolide III or Shh (500 ng/mL) for 24 h. Luciferase activity was determined in cell lysates and normalized to Renilla activity. Each value is expressed as the mean±standard deviation, n=3. *p<0.05 compared to control.

**Atractylenolides Increase Gli Promoter Activity** To investigate whether atractylenolides activate the Gli promoter, MSCs were transfected with a Gli promoter reporter construct and then either left unstimulated or stimulated with increasing concentrations of atractylenolides for 24 h. As shown in Fig. 1, atractylenolides I and III activated the Gli promoter in a dose-dependent manner from 3 to 100 µg/mL, while an increase in Gli promoter activity was also observed with Shh, which used as the positive control.¹⁵ These observations demonstrated that both atractylenolides I and III activate the Gli promoter, thus we focused on further confirming their effects during the differentiation of MSCs into chondrocytes.

**The Effects of Atractylenolides on Gli Promoter Activity Depend on Shh** To assess whether the effects of atractylenolides on Gli promoter activity were mediated by Shh, we tested the effect of the Shh antagonist, cyclopamine, on atractylenolide-mediated Gli promoter activity. The results revealed that atractylenolide I induced an increase in Gli promoter activity, and that atractylenolide III-induced upregulation.
was inhibited by cyclopamine in a dose-dependent manner (Fig. 2a). Similar inhibition of atractylenolide I-mediated Gli promoter activity by cyclopamine is shown in Fig. 2b. Taken together, these results indicate that the effects of atractylenolides on Gli promoter activity were mediated by Shh.

**Atractylenolides Induced the Differentiation of MSCs into Chondrocytes** Because atractylenolides promoted Gli promoter activity in MSCs, we next examined whether atractylenolides induced the differentiation of MSCs into chondrocytes. MSCs were exposed to medium alone or atractylenolides (3, 30 µg/mL) for 7 d, and total cellular RNA was then isolated. Using Q-RT-PCR, we found that both atractylenolides I and III treatment resulted in a dose-dependent increase in the expression of chondrocyte marker genes, including collagen II and aggrecan (Figs. 3a, b). To confirm the effects of atractylenolides on the expression of chondrocyte markers, we measured collagen II and aggrecan protein levels in MSCs treated with or without atractylenolides by Western blot analysis. As shown in Fig. 3c, both atractylenolides induced a strong upregulation of collagen II and aggrecan protein levels. Immunocytochemical techniques showed that both atractylenolides induced a significant increase in collagen II and aggrecan positive cells relative to the controls (Fig. 3d). In conclusion, both atractylenolides I and III induced the differentiation of MSCs into chondrocytes.

**Shh-Gli Pathways Were Activated by Atractylenolides during the Differentiation of MSCs** Based on the effects of atractylenolides on Gli promoter activity and osteoblastic differentiation described above, we postulated that atractylenolides may act to potentiate Shh signaling. To investigate this possibility, we examined the expression of the Shh target gene, Gli-1, and the Shh gene itself, during the differentiation of MSCs. MSCs were treated with atractylenolides and total RNA was then isolated. The mRNA levels of Shh and Gli-1 were analyzed by Q-RT-PCR. As shown in Figs. 4a, b, the levels of Shh and Gli-1 mRNA increased in a dose-dependent manner in MSCs treated with atractylenolides. In order to determine the effects of atractylenolides on the levels of Shh protein signaling during differentiation of MSCs, Western blot analysis was carried out. The results demonstrated that atractylenolides increased the levels of Shh and its target gene, Gli-1 (Fig. 4c), suggesting that Shh-Gli pathways were activated by atractylenolides during the differentiation of MSCs.

**The Effects of Atractylenolide III on the Differentiation of MSCs Are Dependent on the Shh Pathway** Because Shh signaling was activated by atractylenolides in MSCs, we next investigated the contribution of this signaling to the prodifferentiation effects of atractylenolide III. The Shh inhibitor, cyclopamine, was used for these studies. The effects of atractylenolides alone on the stimulation of Sox9, collagen II and aggrecan mRNA expression were observed. When MSCs were stimulated with 500 nM cyclopamine in the absence or presence of atractylenolides, cyclopamine inhibited the atractylenolide-induced expression of Sox9 and chondrogenic marker mRNAs (Fig. 5a). Moreover, Western blot analysis showed that cyclopamine blocked the atractylenolide-induced elevation of Sox9 and chondrogenic marker protein levels (Fig. 5b). Therefore, the prodifferentiation effects of atractylenolides were dependent on Shh signaling.

![Graph](https://via.placeholder.com/150)

**Fig. 2. The Effects of Atractylenolides on Gli Promoter Activity Are Dependent on Shh**

(a) Cyclopamine inhibited the atractylenolide III-induced Gli promoter activity. (b) Cyclopamine inhibited the atractylenolide I-induced Gli promoter activity. MSCs were transfected with a Gli promoter reporter construct and stimulated with the indicated concentrations of cyclopamine (0–500 nM) in the absence or presence of atractylenolides I or III (30 µg/mL). Luciferase activity was determined in cell lysates and normalized to Renilla activity. Each value is expressed as the mean ± standard deviation, n = 3. *p < 0.05 compared to control (-tractylenolide).

**DISCUSSION**

**In vitro** differentiation of MSCs into chondrocytes provides a useful experimental system in which to study stem cell fate, and which is also of therapeutic potential. We hypothesized that a functional cell-based assay based on Shh pathway would be a powerful tool, because it would enable the identification of compounds that affect this incompletely understood differentiation process. First, in MSCs transfected with the Gli promoter, which is the target gene of the Shh pathway, both atractylenolides III and I markedly increased Gli promoter activity. Second, atractylenolide was identified by its ability to induce the differentiation of MSCs into chondrocytes in a phenotypic cellular assay. Third, we have provided evidence to show that Shh signaling is involved in the action of atractylenolide. This structure-function relationship between atractylenolides and Shh provides a new strategy for the screening of drugs that control stem cell fate.

An important finding in the current study was that we identified atractylenolide by phenotypic cellular analysis, which showed that it induced the differentiation of MSCs into chondrocytes. Under appropriate conditions, MSCs can differentiate into all mesodermal cell types such as osteoblasts, chondrocytes, myoblasts, stromal cells and skeletal muscle cells. Therefore, MSCs were used in the cell-based assay in this study. The results from immunohistochemistry, western blotting and real-time PCR analysis illustrated that atractylenolides induced MSCs to differentiate into chondrocytes. This effect is consistent with results showing that atractylenolides remarkably increased the expression of the cartilage related transcription factor, Sox9. Sox9 contains a high mobility
group domain and is believed to be an essential master gene for the formation of the cartilaginous skeleton, because mutations in human and mouse Sox9 alter cartilage differentiation. Furthermore, expression of Sox9 is required for the activation of cartilage markers such as the collagen II gene. We found that upregulation of Sox9 by atractylenolides during in vitro differentiation of MSCs promotes the expression of more specific chondrogenic markers. Our results are in agreement with a previous report showing that Sox9 upregulation is essential for chondrogenesis. Sox9 may therefore be a downstream mediator of atractylenolides during the promotion of chondrogenesis in MSCs.

The findings described above prompted us to investigate whether Shh signaling was activated by atractylenolide during the differentiation of MSCs. Further support for the hypothesis that atractylenolide may regulate Shh signaling was obtained by examining the expression of Shh and its target gene, Gli. The mechanism of action of atractylenolide on the expression of Shh and Gli-1 is presumably transcriptional, because atractylenolide increases Shh and Gli expression at both the mRNA and protein levels, as shown by real-time RT-PCR and western blotting, respectively. The dose-dependent upregulation of Gli-luciferase activity by atractylenolide provided further evidence that upregulation of Gli-1 is achieved at the
transcriptional level. In these experiments, Shh was used as the positive control because it is the most effective stimulator of Gli promoter activity. Shh was also active in this assay. Atractylenolide III, which possesses –OH at the 8-position, markedly increased Gli promoter activity in a dose-dependent manner. However, atractylenolide I, which lacks of the –OH at the 8-position, was also effective in promoting Gli promoter activity. These results suggested that the –OH at the 8-position of atractylenolide III is not essential for its effect. Given that Gli expression is considered as a marker of Shh pathway activity, the fact that Gli was upregulated by atractylenolide verified that Shh signaling is activated by atractylenolide during the differentiation of MSCs.

Fig. 4. Shh-Gli Pathways Were Activated by Atractylenolides during the Differentiation of MSCs
(a,b) Shh-Gli pathways were activated by atractylenolides as indicated by Q-RT-PCR analysis for Shh and Gli-1 mRNA. MSCs were exposed to medium alone, atractylenolides (3 or 30 µg/mL) for 7d, and total RNA was prepared for Q-RT-PCR analysis. (c) Shh-Gli pathways were activated with atractylenolides as indicated by Western blot analysis. MSCs were exposed to medium alone, atractylenolides (3 or 30 µg/mL) for 7d, and the cells were lysed for Western blot analysis (upper). Comparison of the densities of Shh, Gli-1/actin bands among the experimental groups after atractylenolide treatment is shown (lower). *p<0.05 compared to control (0 µg/mL). Each value is expressed as the mean±standard deviation, n=3.

Fig. 5. The Effects of Atractylenolides on the Differentiation of MSCs Are Dependent on Shh Pathways
(a) Cyclopamine inhibited the expression of atractylenolide-induced Sox9 and chondrogenic marker mRNAs. MSCs were stimulated with 500 nM of cyclopamine in the absence or presence of atractylenolides (30 µg/mL) for 7d, and total RNA was prepared for Q-RT-PCR analysis. (b) Cyclopamine inhibited the expression of atractylenolide-induced Sox9 and chondrogenic markers. MSCs were stimulated with 500 nM of cyclopamine in the absence or presence of atractylenolides (30 µg/mL) for 7d, and the cells were lysed for Western blot analysis (upper). Comparison of the density of Sox9, collagen II and aggrecan/actin bands among the experimental groups after atractylenolide treatment is shown (lower). Each value is expressed as the mean±standard deviation, n=3. *p<0.05 compared to control (vehicle).
from positively regulating Gli promoter activity, chondrogenic markers and Sox9 expression. These results demonstrated that the effects of atractylenolide on chondrogenic differentiation in MSCs are dependent on Shh signaling.

The results we obtained are promising for various clinical applications. First, osteoarthritis, one of the most common bony diseases, is considered to be caused by an imbalance in cartilage homeostasis during the aging process. Cell therapy based on the chondrogenic differentiation of MSCs is an attractive strategy for treating osteoarthritis. Our analysis of atractylenolides illustrated that simple supplementation of such small molecules can enhance the yield of chondrocytes from MSCs. This effect was not dependent on the scale of the culture, because a similar induction by atractylenolide was seen on larger dishes. This means that treatment of MSCs with atractylenolide may facilitate the large-scale generation of chondrocytes, which is one of the major barriers to their use. Second, it is well known that the clinical use of large molecules (e.g., therapeutic antibodies or trophic factors) is currently limited by cost and the difficulties in delivery of concentrated recombinant proteins. However, atractylenolides are small molecules, and they may potentially be employed as Shh stimulators in clinical applications. The identification of small molecules that enhance Shh signaling may open the door to new therapeutic strategies. Our studies suggest that atractylenolides are candidate molecules for such strategies. In addition, the discovery that pharmacological modulation of Shh signaling can enhance chondrogenic differentiation provides an avenue for potential therapeutic development. Third, because Shh protein provides beneficial effects in the treatment of Parkinson’s disease, regulation of Shh signaling by atractylenolide may provide a new approach to the treatment of brain diseases.

In conclusion, our data demonstrate that a cell-based assay system based on Shh signaling can be used to identify molecules that induce the differentiation of MSCs into chondrocytes, and that atractylenolides markedly increase the efficiency of chondrocyte differentiation. These findings may contribute to the efficient production of chondrocytes from cultured MSCs for many applications in the field of bone and joint repair, and raise interesting questions about the role of atractylenolides in chondrogenesis.

Acknowledgments This work was supported by the National Nature Science Foundation of China (30772861), the “Hendeca-Five” Technology Pedestal Planning Project of China (2008BA153B074), and the Great Project of Technology Achievement Transformation of Higher Education in Guangdong (cgzhzdl009).

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