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Fluvastatin and Lovastatin but not Pravastatin Induce Neuroglial Differentiation in Human Mesenchymal Stem Cells

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Abstract Recent studies have shown that statins, the most potent inhibitors of 3-hydroxy-2-methylglutaryl coenzyme A (HMG-CoA) reductase, stimulate bone formation in vitro and in rodents by activating the expression of bone morphogenetic protein-2 (BMP-2), one of the most critical osteoblast differentiation-inducing factors. However, the effect of statins on mesenchymal stem cells (MSCs) is yet to be reported. The purpose of this study is to investigate the influence of fluvastatin, lovastatin, and pravastatin, three commonly prescribed lipid-lowering agents, on the proliferation and differentiation of human MSCs. To our surprise, even though fluvastatin and lovastatin effectively suppressed the growth of human MSCs, a neuroglia rather than osteoblast-like morphology was observed after treatment. Interestingly, such morphological change was inhibited by the co-addition of geranylgeranyl pyrophosphate (GGPP). Immunofluorescence staining with antibodies against neuron-, astrocyte-, as well as oligodendrocyte-specific markers confirmed the neuroglial identity of the differentiated cells. However, BMP-2 is unlikely to play a positive role in neuroglial differentiation of MSCs since its expression was down-regulated in fluvastatin-treated cells. Taken together, our results suggest that fluvastatin and lovastatin induce neuroglial differentiation of human MSCs and that these cholesterol-lowering agents might be used in conjunction with MSC transplantation in the future for treating neurological disorders and injuries. J. Cell. Biochem. 93: 917–928, 2004.

Key words: mesenchymal stem cells; statins; neuroglia

Inhibitors of 3-hydroxy-2-methylglutaryl coenzyme A (HMG-CoA) reductase, or statins, have been described as the most effective class of drugs to reduce serum cholesterol levels [Veillard and Mach, 2002]. These agents lower cholesterol production by inhibiting the conversion of HMG-CoA to mevalonate, a rate-limiting step in the pathway of cholesterol biosynthesis [Bauer, 2003]. It has been reported that statins are beneficial on the coronary endothelium in patients with coronary artery disease [Treasure et al., 1995]. Reduced recurrence rates and improved clinical outcome after coronary stent implantation in patients with statin therapy have also been demonstrated [Walter et al., 2000]. In addition, coronary endothelial vasomotor function has been shown to be improved by statin therapy [Vita et al., 2000] which might be attributed to an accelerated re-endothelialization of damaged cardiac tissues involving the mobilization and incorporation of bone marrow-

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derived endothelial progenitor cells [Walter et al., 2002].

The biologic effects of statins on bone metabolism have also been reported and it has been found that statins are potent stimulators of bone formation [Mundy et al., 1999]. In fact, several recent studies have shown that statins induce expression of the gene encoding bone morphogenetic protein-2 (BMP-2), one of the most crucial activators for osteoblast differentiation in the in vitro cell culture and in animal bone defect models, respectively [Sakou, 1998; Sugiyama et al., 2000]. Moreover, increased BMP-2 expression in human osteosarcoma and murine embryonic stem cells as well as an enhanced formation of mineralized nodules at a later stage of culture by mevastatin has also been reported [Phillips et al., 2001]. Taken together, these data demonstrated unequivocally the efficacy of statins in inducing osteoblastic differentiation of various types of cells. However, the effects of statins on the proliferation and/or differentiation of mesenchymal stem cells (MSCs), the progenitors of osteoblasts, were not reported although an animal experiment showed that simvastatin induces osteoblastic differentiation and inhibits adipocytic differentiation in mouse marrow stromal cells [Song et al., 2003]. The purpose of present study is to investigate the effects of fluvastatin, lovastatin, and pravastatin, three commonly prescribed lipid-lowering agents, on the proliferation and differentiation of MSCs based on the hypothesis that statins might suppress the proliferation but stimulate the osteogenic differentiation of bone marrow-derived MSCs.

MATERIALS AND METHODS

Reagents

Human recombinant basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and bone morphogenetic factor-2 (BMP-2) were purchased from R&D Systems (Minneapolis, MN). Antibodies against human microtubule-associated protein-2 (MAP-2), βIII-tubulin, and glial fibrillary acidic protein (GFAP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody against human oligodendrocyte marker O4 was obtained from Chemicon (Temecula, CA). Antibody against human neuron specific enolase (NSE) was purchased from Dako (Carpinteria, CA). Secondary antibody conjugated with fluorescein isothiocyanate (FITC) or rhodamine was obtained from Santa Cruz Biotechnology. Geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP) were purchased from Sigma-Aldrich (St. Louis, MO). Cisplatin and doxorubicin were purchased from David Bull Laboratory (Victoria, Australia) and Pharmacia Corp. (Piscataway, NJ), respectively. Oligonucleotide primers used for PCR analysis were synthesized by MDBio (Taipei, Taiwan). Fluvastatin, lovastatin, and pravastatin were gifts from Standard Chemical and Pharmacy Co., Ltd., Taiwan.

Preparation and Characterization of Human Bone Marrow MSCs

Human bone marrow MSCs were isolated according to a method described previously by us [Lee et al., 2004] with slight modification. Briefly, bone marrow was collected during orthopedic surgeries with informed consent. Mononuclear cells were harvested by negative immuno-depletion of CD3, CD14, CD19, CD38, CD66b, and glycophorin-A positive cells using a commercially available kit (RosetteSep®, StemCell Technologies, Vancouver, BC, Canada) as per manufacturer's instructions, followed by Ficoll-Paque (Amersham-Pharmacia, Piscataway, NJ) density gradient centrifugation ($1.077 g/cm^3$), and were plated in non-coated tissue culture flasks (Becton Dickinson, Flanders, NJ) in expansion medium. Cells were allowed to adhere overnight and non-adherent cells were discarded and medium changes were carried out twice weekly thereafter. Expansion medium consists of Iscove's modified Dulbecco's medium (IMDM, Gibco BRL, Grand Island, NY) and 20% fetal bovine serum (FBS, Hyclone, Logan, UT) supplemented with 10 ng/ml bFGF, 100 U penicillin, 1,000 U streptomycin, and 2 mM L-glutamine (Gibco BRL). To obtain single cell-derived, clonally-expanded MSCs, the isolated plate-adhering second-passage cells were serially diluted and plated onto 96-well plates (Becton Dickinson) in expansion medium at a final density of 0.3 cell/well (30 cells for each 96-well plate). Cell pellets in the wells were obtained and the most rapidly proliferating clone was selected. Surface phenotyping was performed using flow cytometry. Antibodies against human antigens CD7, CD14, CD16, CD19, CD29, CD33, CD34, CD38, CD44, CD45, CD73, CD90, CD105, CD117, CD127, CD135, CD166, HLA-ABC, and HLA-DR were purchased from Becton Dickinson. Antibody against human antigen

CD133 was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Antibody against human antigen SH-2 was purified from SH-2 hybridoma cell line (American Type Culture Collection, Rockville, MD). In essence, MSCs were detached and stained with fluorescein- or phycoerythrin-coupled antibodies and analyzed with FACS Calibur (Becton Dickinson). MSCs were routinely maintained in IMDM supplemented with 10% FBS, 10 ng/ml bFGF, and 10 ng/ml EGF at 37°C in 5% CO_2.

Cell Viability Assay

MSCs were seeded at a density of 3,000 cells per well in a 96-well plate. After overnight incubation, cells were replenished with media containing 10, 1, or 0% FBS with varying concentrations of fluvastatin or pravastatin. Forty-eight hours later, the viability of MSCs was determined by MTS [3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] assay using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay kit (Promega, Madison, WI). Briefly, 20 μl of a solution containing MTS/PMS (phenazine methosulfate, an electron coupling reagent) (20:1 V/V) was added to each well and the plates were incubated at 37°C for 4 h before the optical density of the soluble formazan produced in each well being measured at 490 nm by a microplate reader (PerkinElmer, Boston, MA). Each experiment was performed at least twice in triplicate.

Immunofluorescence Staining

MSCs cultured overnight on the coverslips were treated with 50 μM fluvastatin in 10% FBS for 48 h. For immunofluorescence staining, cells were fixed with 4% paraformaldehyde in PBS (Gibco BRL) for 15 min, permeabilized with 0.1% Triton X-100 for 15 min, and then blocked with 5% BSA in PBS for 30 min. Coverslips were then incubated respectively with primary antibodies against human MAP-2 (1:100), human NSE (1:100), human βIII-tubulin (1:100), human O4 (1:100), and human GFAP (1:100) at room temperature for 1 h and followed by either FITC- or rhodamine-labeled secondary antibody (1:100). One hour later, cells were counterstained with 1 μg/ml Hoechst 33258 for 30 min and then washed with PBS, mounted in 70% glycerol, and observed with fluorescence optics on an Olympus BX50 microscope (Tokyo, Japan).

RT-PCR Analysis

Total RNAs were isolated from MSCs after they were incubated with 50 μM fluvastatin for 48 h using Ultraspec™-II RNA Resin Purification System (Biotecx Laboratories, Inc., Houston, TX). The synthesis of first strand cDNA was performed using 5 μg total RNAs as templates in a 20 μl reaction mixture containing 0.4 mM dNTP, 12.5 μM oligodT, 12.5 μM random hexamer, and 200 U reverse transcriptase (New England Biolabs, Inc., Beverly, MA). The reverse transcription was done in a block incubator (Primus-25, MWG Biotech Ltd., Ebersberg, Germany) for 60 min at 42°C after heating at 70°C for 5 min. PCR amplification was carried out in a reaction mixture containing 250 ng of cDNA, 0.4 mM dNTPs, 1 U of polymerase, and 200 pM of the following primers: BMP-2-specific: sense, 5′-GAGTTGCGGCTGCTCAGCATGTT-3′, and antisense, 5′-ACATGTCTCTTGGAGACACCT-3′; and GAPDH-specific: sense, 5′-GACCACAGTCCATGCCATCAC-3′, and antisense, 5′-TCCACCACCCTGTTGCTGTAG-3′. RT-PCR of mRNA encoding human BMP-2 and GAPDH resulted in products of 572 and 453 bp, respectively. The PCR cycles consisted of 1 min initial denaturation at 94°C, followed by 30 cycles of 94°C for 45 s, 59°C for 45 s, and 72°C for 1 min, and 7 min final elongation at 72°C in a thermal cycler (Primus-25, MWG Biotech Ltd.). Ten microliters of each PCR products were separated in a 1% agarose gel and photographed under UV light.

Statistical Analysis

Each data point in the figures represents the mean ± SD for three individual determinations. Statistically significant difference was set at P value < 0.05 by two-tailed Student's t-tests.

RESULTS

Clonally-expanded MSCs were isolated from human bone marrow and characterization by flow cytometry. As shown in Table I, these cells were negative for CD7 (gp40), CD14 (LPS-R), CD16 (FcγRIIIa), CD19 (B4), CD33 (P67), CD34 (gp105-120), CD38 (T10), CD45 (leukocyte common antigen), CD127 (IL-7Rα), CD133 (AC133), CD135 (Flt-3), and HLA-DR. On the other hand, these cells were positive for CD29 (β1-integrin), CD44 (hyaluronate receptor), CD73 (ecto-5-nucleotidase), CD90 (Thy-1), CD105 (endoglin), CD166 (ALCAM), HLA-

TABLE I. Surface Phenotype of BM–Mesenchymal Stem Cells (MSCs)

Positive (+)	Negative (−)
CD29	CD7
CD44	CD14
CD73	CD16
CD90	CD19
CD105	CD33
CD166	CD34
SH-2	CD38
HLA-ABC	CD45
	CD117
	CD127
	CD133
	CD135
	HLA-DR

ABC, and most importantly, MSC-specific antigen SH-2.

To examine the response of MSCs to different statins as well as the influence of serum on such response, cells were incubated with varying concentrations of fluvastatin and pravastatin in the presence of 10, 1, or 0% FBS for 48 h and their viability was subsequently determined by MTS assays. In the presence of serum, dose-dependent growth suppression of MSCs by fluvastatin was observed (Fig. 1A,B), but significant inhibition by pravastatin was not detected until 250 μM of this drug was added. Under serum-free condition, as low as 0.016 μM of fluvastatin was able to suppress MSCs growth, whereas no apparent growth inhibition was found even when cells were incubated with 250 μM of pravastatin (Fig. 1C).

To assess whether differentiation was accompanied with reduced proliferation in MSCs caused by fluvastatin, cells were subjected to microscopic examination after they were incubated with 50 μM of fluvastatin for 24 h. Surprisingly, some of the MSCs showed a stellate cell body with many dendrite-like protrusions (Fig. 2C) and almost all of them exhibited this neuroglia-like morphology 48 h after drug

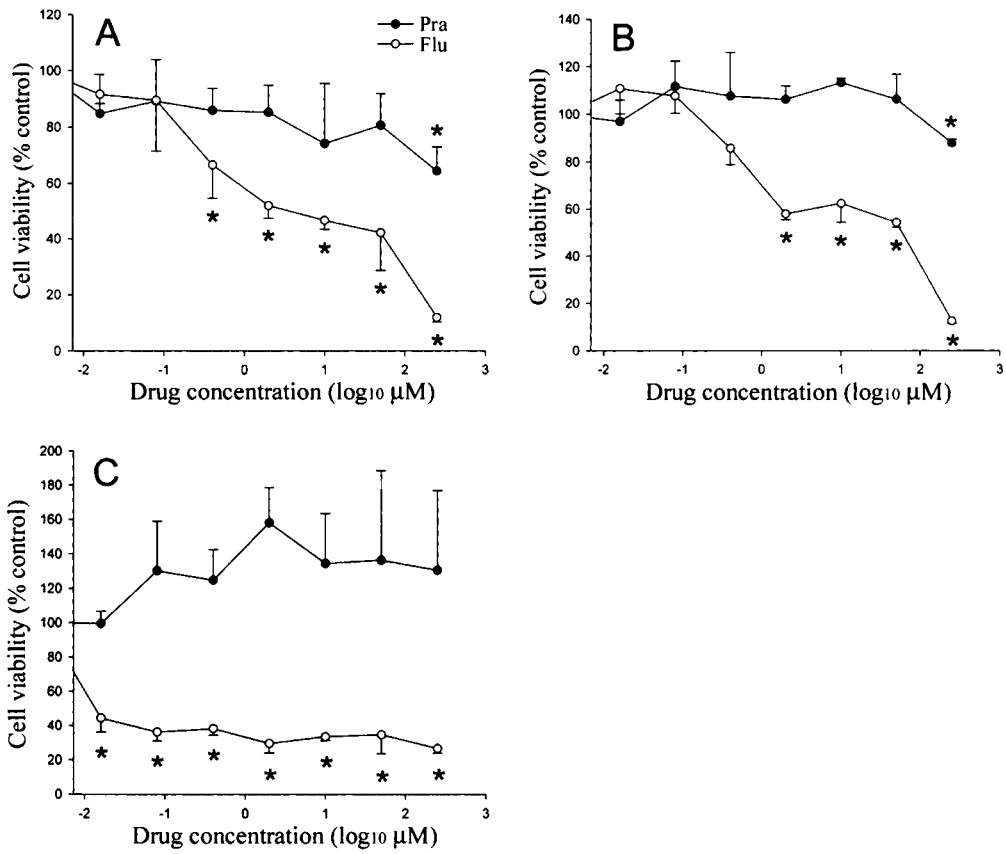

Fig. 1. Effects of fluvastatin and pravastatin on the viability of human mesenchymal stem cells (MSCs) cultured in medium containing different concentrations of fetal bovine serum (FBS). Viability of human MSCs after being incubated with varying concentrations (0.016, 0.08, 0.4, 2, 10, 50, and 250 μM) of fluvastatin or pravastatin in a medium containing 10 (**A**), 1 (**B**), or 0% (**C**) of FBS for 48 h was determined by MTS assay. The results are expressed as mean ± SD of triplicates. $*P < 0.05$ versus control according to *t*-test.

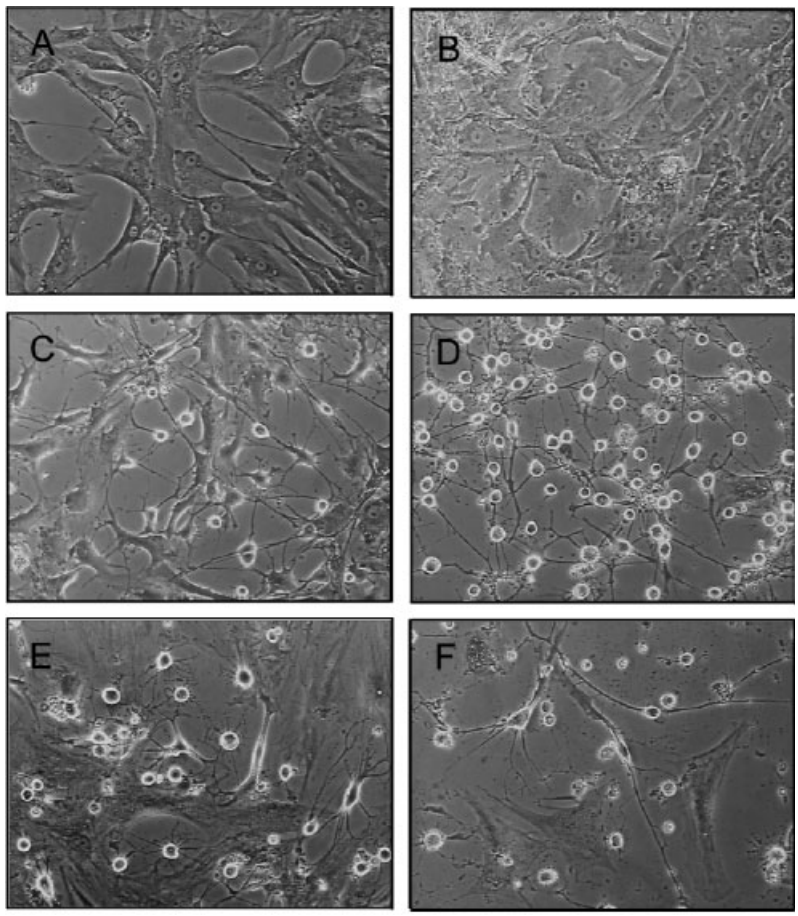

Fig. 2. Morphological changes of human MSCs induced by fluvastatin. Cells were plated at a density of 6×10^3 cells/cm^2 onto 24-well plates. Sixteen hours later, culture medium was replenished by the one containing either 10% FBS (**A** and **B**), 10% FBS plus 50 μM fluvastatin (**C** and **D**), or 1% FBS plus 2 μM drug (**E** and **F**). Photographs were taken 24 (A, C, and E) or 48 h (B, D, and F) after drug treatment. (Magnification, ×100.)

treatment (Fig. 2D). Induction of the morphological change in MSCs could be achieved with lower concentration of fluvastatin (2 μM) when serum level was reduced to 1% (Fig. 2E,F). Interestingly, neuroglia-like morphology of MSCs was also observed when fluvastatin was replaced by lovastatin (Fig. 3), but not by pravastatin (data not shown). To determine whether the morphological change in MSCs induced by two statins was a mere stress response to the cytotoxic effects of these agents, cells were cultured in the presence of two cytotoxic drugs, cisplatin and doxorubicin. While severe shrinkage of MSCs was detected when they were incubated in the presence of either cisplatin (10 μg/ml) or doxorubicin (1 μg/ml) for 2 and 4 days, respectively, no neuroglial-like morphology in these cells was observed (Fig. 4).

To determine the identity of these morphologically-changed MSCs, immuno fluorescence stainings were carried out with antibodies against three neuron-specific markers including MAP-2, βIII-tubulin, and NSE. As can be seen in Figure 5, the majority of cells expressed these neuron-specific proteins. Expression of the astrocyte-specific marker, GFAP, and the oligodendrocyte-specific antigen, O4, in these cells was also analyzed. Only morphologically changed MSCs were stained positively by either antibody (Fig. 6D,H).

To elucidate the molecular mechanism underlying MSC differentiation induced by fluvastatin, we examined the involvement of protein isoprenylation in this process since earlier studies have shown good correlation between the inhibition of isoprenylation of several small GTPases and the activation of genes whose

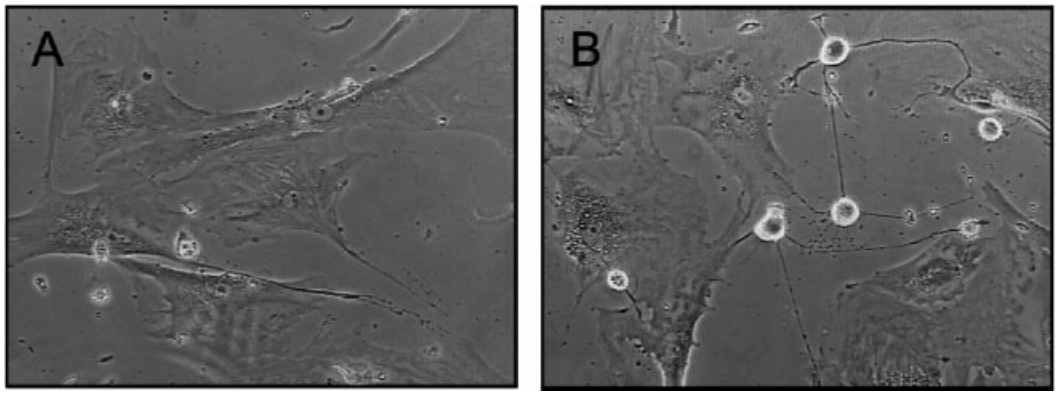

Fig. 3. Morphological changes of human MSCs induced by lovastatin. Cells were seeded in a medium containing 10% FBS and treated with drug as described in Figure 2 except 50 μM lovastatin was used to replace fluvastatin (**B**). (Magnification, ×100.)

products could facilitate differentiation [Martin et al., 2001; Ohnaka et al., 2001; Maeda et al., 2003]. Interestingly, morphological change of MSCs induced by fluvastatin was suppressed by the addition of GGPP, but not by FPP (Fig. 7). We then assessed the role of bone morphogenetic factor (BMP-2), a cytokine with neuronal differentiation-promoting activity [Pisano et al., 2000; Yung et al., 2002] whose expression can be stimulated by various statins [Mundy et al.,

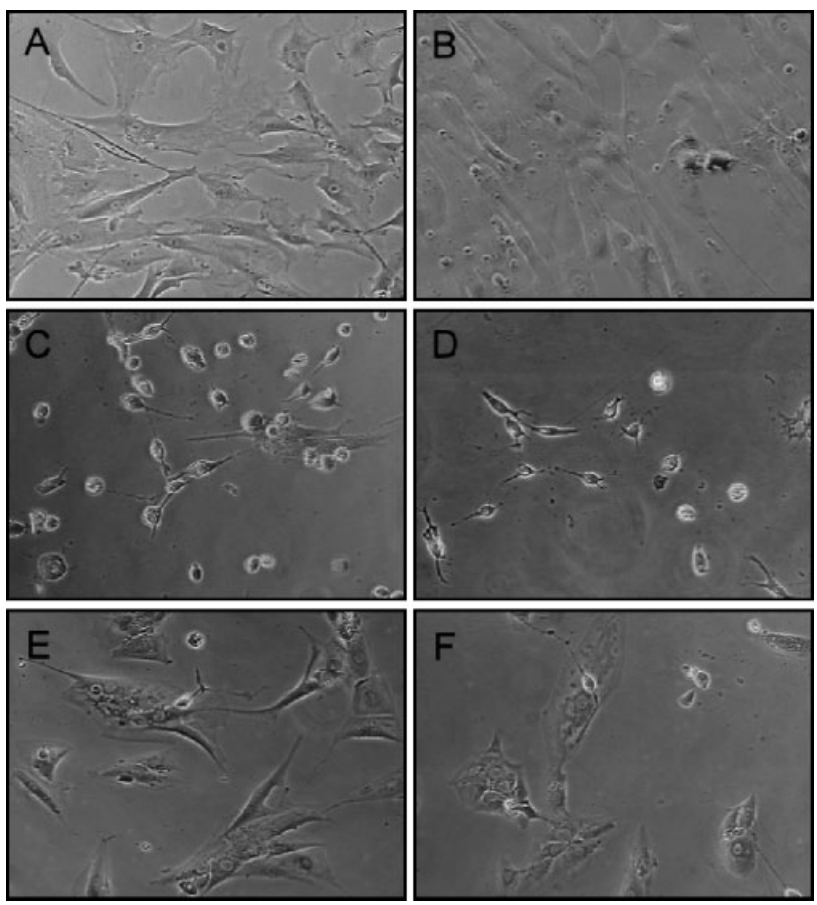

Fig. 4. Morphological changes of human MSCs resulted from cisplatin and doxorubicin treatment. Cells were incubated without (**A** and **B**) or with 10 μg/ml of cisplatin (**C** and **D**) or 1 μg/ml of doxorubicin (**E** and **F**) for 2 (A, C, and E) or 4 (B, D, and F) days before photographs were taken. (Magnification, ×200.)

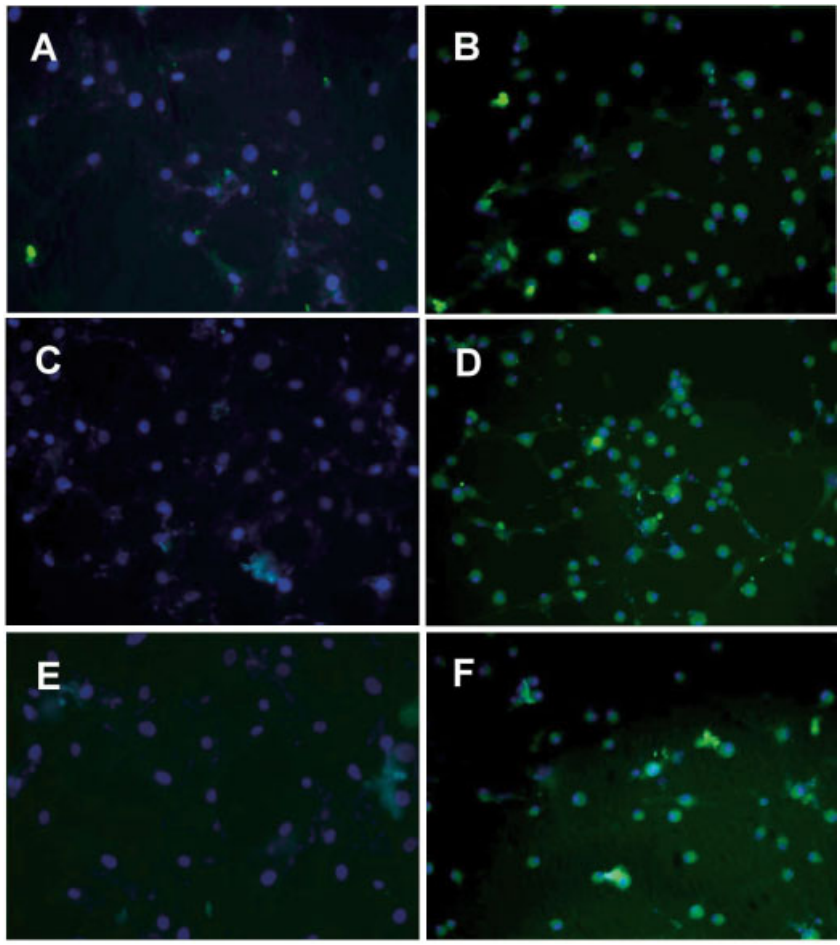

Fig. 5. Expression of neuron-specific markers on human MSCs induced by fluvastatin. Cells were seeded onto coverslips and processed as described in the ''Materials and Methods'' after being incubated without (**A**, **C**, and **E**) or with (**B**, **D**, and **F**) 50 μM fluvastatin. Forty-eight hours later, cells were stained with anti-MAP-2 (A and B), anti-neuron specific enolase (NSE) (C and D), or anti-βIII-tubulin (E and F) antibody, respectively. After counter staining the nuclei with Hoechst 33258, cells were examined under a fluorescence microscope. (Magnification, ×200.) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

1999; Sugiyama et al., 2000; Ohnaka et al., 2001; Song et al., 2003; Maeda et al., 2004], in neuroglial differentiation of MSCs by RT-PCR analysis. To our surprise, a down-regulated BMP-2 expression was found in fluvastatin-treated cells (Fig. 8). Accordingly, an osteoblastic-like morphology of MSCs was detected 7 days after they were incubated with the aforementioned cytokine (Fig. 9).

DISCUSSION

Clonally-expanded MSCs from human bone marrow were obtained and their phenotype was characterized by flowcytometric analysis. Profile of the molecules expressed on cell surface (Table I) is in good agreement with the ones reported earlier by others [Pittenger et al., 1999], indicating their authenticity as MSCs. We then examined the response of these cells to statins because some of the cholesterol-lowering agents have been shown to induce osteogenesis [Mundy et al., 1999] plus our own observation that these MSCs could differentiate into osteoblasts [Lee et al., 2004]. MTS assays demonstrated that not only was the growth of MSCs suppressed by fluvastatin but the suppression was in a dose-dependent manner when they were cultured in the presence of FBS. By contrast, pravastatin even at a concentration of 250 μM showed little or no inhibition on MSC growth (Fig. 1). Since certain statins have been

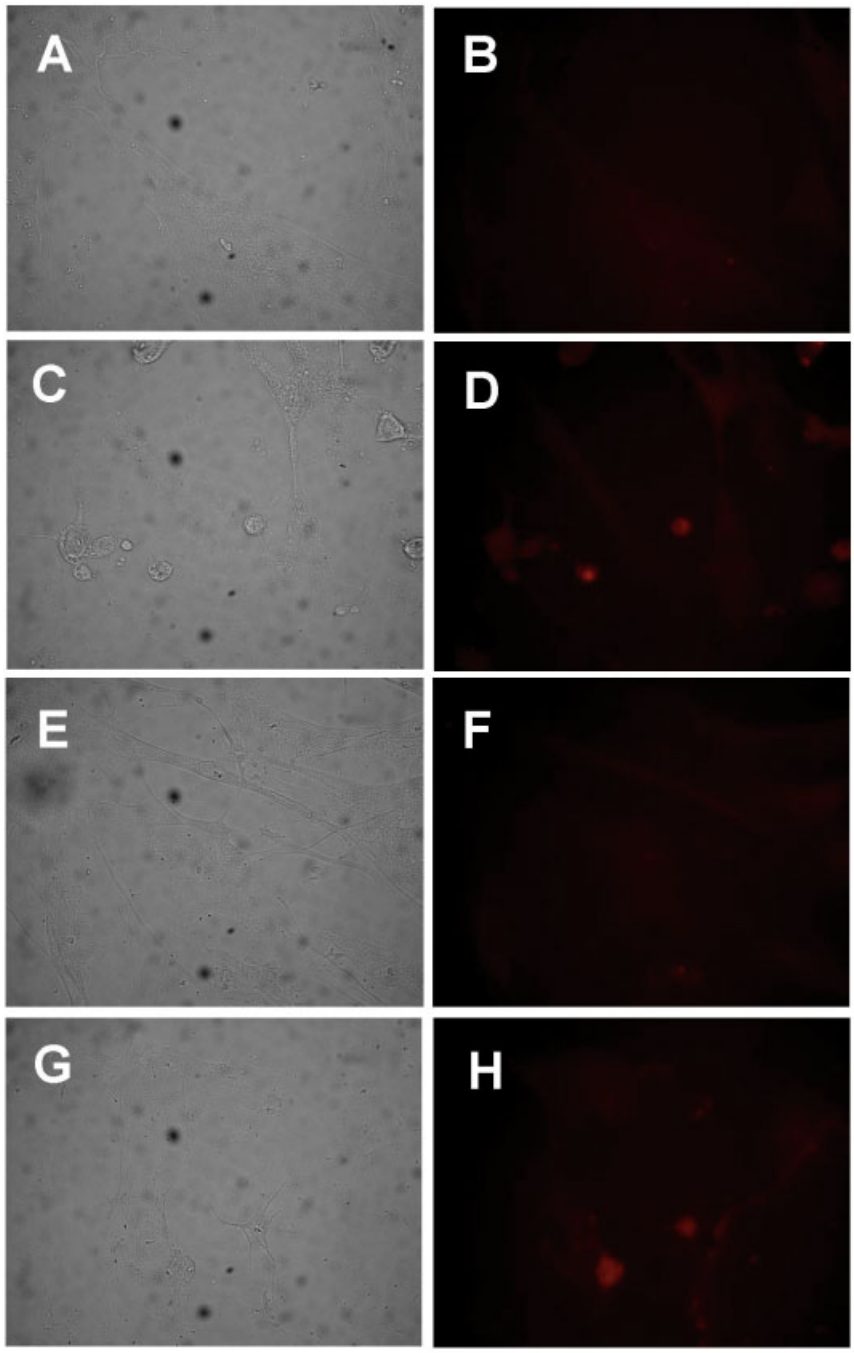

Fig. 6. Expression of astrocyte- and oligodendrocyte-specific markers on human MSCs induced by fluvastatin. Cells were seeded and treated without (**A**, **B**, **E**, and **F**) with 50 μM fluvastatin (**C**, **D**, **G**, and **H**) as described in Figure 4 except immunofluorescence stainings were performed using either an anti-glial fibrillary acidic protein (GFAP) (A–D) or anti-O4 (E–H) antibody, respectively. Photographs were taken either by bright field (A, C, E, and G) or fluorescent microscopy (B, D, F, and H). (Magnification, ×400.) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

shown to induce osteoblastic differentiation of various types of cells [Sugiyama et al., 2000; Song et al., 2003], morphology of MSCs was examined microscopically after drug treatment. To our surprise, a neuroglia-like morphology was observed when these cells were treated with either fluvastatin (Fig. 2) or lovastatin (Fig. 3). Although death of MSCs was detected when they were incubated with high dose of statins, aforementioned morphological change

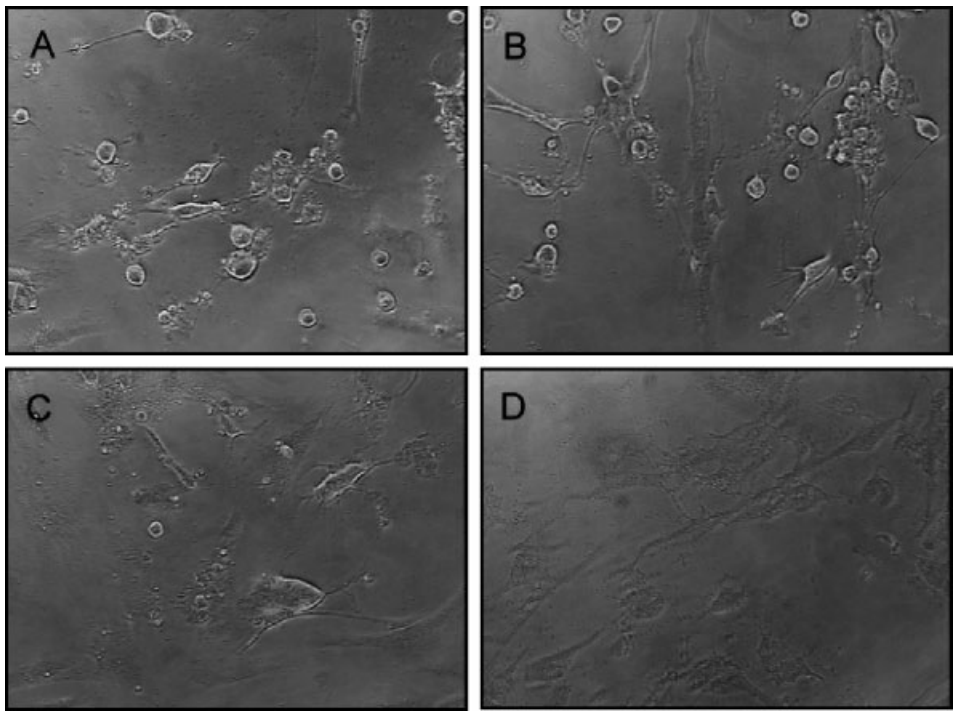

Fig. 7. Morphological change in human MSCs induced by fluvastatin is abolished by geranylgeranyl pyrophosphate (GGPP). Cells were treated with 2 μM fluvastatin in the presence of 5 (**A**) or 10 (**B**) μM of farnesyl pyrophosphate (FPP); or 5 (**C**) or 10 (**D**) μM of GGPP for 48 h before photographs were taken (Magnification, ×100.)

in these cells is unlikely to be accounted by a mere stress response of MSCs to the cytotoxic effects of the cholesterol-lowering agents because no such change could be found when death of these cells was triggered by cytotoxic drugs, cisplatin and doxorubicin (Fig. 4).

While the neuronal identity of these cells was validated by immunofluorescence stainings with antibodies against three neuron-specific markers, MAP-2, βIII-tubulin, and NSE (Fig. 5), their glial cell features were confirmed by the positive stainings by both the anti-GFAP (Fig. 6) and anti-O4 (Fig. 6) antibodies. On the other hand, no apparent morphological change in MSCs was found after pravastatin treatment (data not shown). Intriguingly, an osteoblastic differentiation of mouse bone marrow stromal cells resulted from simvastatin treatment has been reported [Song et al., 2003]. These discrepancies might be attributed to (a) unlike our MSCs were clonally derived, the stromal cells they used were heterogenous that might contain a dominant population of osteoblast precursors which could undergo osteoblastic differentiation under proper circumstances. (b) The inability of simvastatin to stimulate neuroglial differentiation in stromal cells. (c) Species difference [Osyczka et al., 2004]. However, failure of pravastatin in inducing MSCs differentiation reported herein is in agreement with an earlier finding that pravastatin was incapable of triggering osteoblastic differentiation in human osteosarcoma cells [Sugiyama et al., 2000], which might be explained by its poor lipophobicity and cell penetration [Quion and Jones, 1994]. Even though approximately 2 μM of fluvastatin seems to be required to suppress the growth of MSCs, which is higher than the steady-state serum levels (~0.1 μM) needed for hypercholesterolemia therapy [Pan et al., 1990], it is nonetheless within the circulating concentrations (2–4 μM) that were well tolerated [Kornbrust et al., 1989]. Thus, once its long-term effects on MSCs were established, fluvastatin (or lovastatin) might be used as an adjuvant to improve the treatment of neural injury with MSCs as that achieved by the coadministration with several neurotrophic factors [For a review, see Chopp and Li, 2002].

Although the mechanism(s) underlying neuroglial differentiation of human MSCs induced by fluvastatin and lovastatin is unclear at

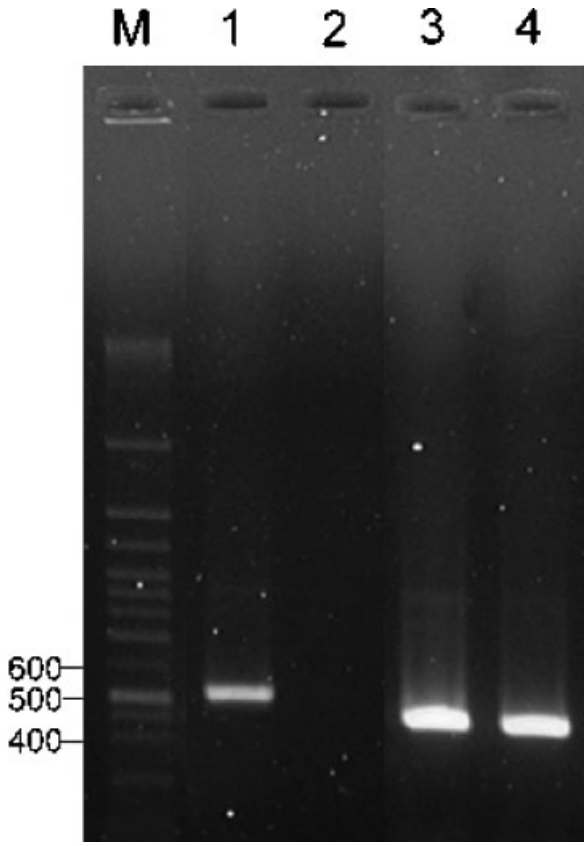

Fig. 8. Bone morphogenetic protein-2 (BMP-2) expression is down-regulated in human MSCs treated with fluvastatin. RT-PCR was carried out as described in the ''Materials and Methods'' using total RNAs prepared from cells incubated in the absence (**lanes 1** and **3**) or presence (**lanes 2** and **4**) of 50 μM fluvastatin for 48 h with BMP-2- (lanes 1 and 2) and GAPDH- (lanes 3 and 4) specific primer sets. Lane M, 100-bp DNA ladders.

present, their inhibitory effects on protein isoprenylation seem to play critical roles because morphological change in these cells induced by fluvastatin was abrogated by the addition of GGPP but not by FPP (Fig. 7), even though both are derived from the metabolism of mevalonate and both can be utilized for protein prenylation. Interestingly, several earlier studies have demonstrated correlations between the inhibition of prenylation in small Rho GTPases (Rho, Rac, and Cdc42) and the activated expression of genes whose products might facilitate cell differentiation. For example, inhibition on Rho-associated kinase might be the reason for pitavastatin-stimulated up-regulation of BMP-2 and osteocalcin expression in primary cultured human osteoblasts [Ohnaka et al., 2001]. Furthermore, statin-induced increases in the apolipoprotein A-I mRNA levels in human HepG2 cells [Martin et al., 2001] and in the VEGF transcript levels in MC3T3-E1 cells [Maeda et al., 2003] have been reported to be abolished by the addition of mevalonate or GGPP.

Regarding the molecule(s) that modulates the neuroglial differentiation of MSCs triggered by statins, BMP-2 is considered to be a good candidate not only because its expression can be induced by various statins but also its efficacy in promoting neuronal differentiation of sympathetic and enteric precursor cells as well as potentiating the elaboration of striatal [Pisano et al., 2000] and cerebral cortical GABAergic neurons [Yung et al., 2002]. However, the involvement of this cytokine in statin-induced neuroglial differentiation of human MSCs was ruled out by our findings that BMP-2 expression in these cells was down-regulated by fluvastatin (Fig. 8) plus an osteoblastic-like morphology of MSCs was clearly detected when they were cultured in the presence of exogenous BMP-2 (Fig. 9). More work is needed to elucidate the

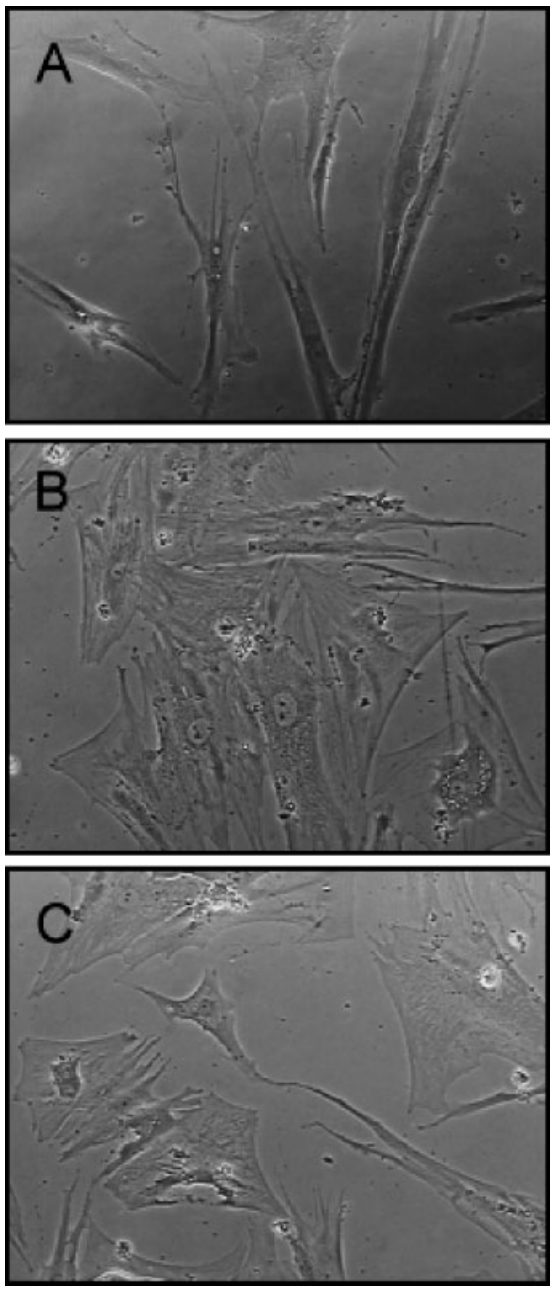

Fig. 9. Morphological change of human MSCs induced by exogenous BMP-2. Cells were seeded in 12-well plates 24 h before medium was replaced by the one without FBS. Photographs were taken 7 days after cells were incubated without (**A**) or with 100 (**B**) or 500 (**C**) ng/ml of BMP-2. (Magnification, ×200.)

molecular mechanism(s), especially the involvement of other neurotrophic factors, leading to the neuroglial differentiation of human MSCs induced by statins. Experiments of this nature are currently undergone in our laboratory.

REFERENCES

Bauer DC. 2003. HMG CoA reductase inhibitors and the skeleton: A comprehensive review. Osteoporos Int 14:273–282.

Chopp M, Li Y. 2002. Treatment of neural injury with marrow stromal cells. Lancet Neurol 1:92–100.

Kornbrust DJ, MacDonald JS, Peter CP, Duchai DM, Stubbs RJ, Germershausen JI, Alberts AW. 1989. Toxicity of the HMG-coenzyme A reductase inhibitor, lovastatin, to rabbits. J Pharmacol Exp Ther 248:498–505.

Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. 2004. Isolation of multi-potent mesenchymal stem cells from umbilical cord blood. Blood 103:1669–1675.

Maeda T, Kawane T, Horiuchi N. 2003. Statins augment vascular endothelial growth factor expression in osteoblastic cells via inhibition of protein prenylation. Endocrinol 144:681–692.

Maeda T, Matsunuma A, Kurahashi I, Yanagawa T, Yoshida H, Horiuchi N. 2004. Induction of osteoblast differentiation indices by statins in MC3T3-E1 cells. J Cell Biochem 92:458–471.

Martin G, Duez H, Blanquart C, Berezowski V, Poulain P, Fruchart JC, Najib-Fruchart J, Glineur C, Staels B. 2001. Statin-induced inhibition of the Rho-signaling pathway activates PPARalpha and induces HDL apoA-I. J Clin Invest 107:1423–1432.

Mundy G, Garrett R, Harris S. 1999. Stimulation of bone formation in vitro and in rodents by statin. Science 286: 1946–1949.

Ohnaka K, Shimoda S, Nawata H, Shimokawa H, Kaibuchi K, Iwamoto Y, Takayanagi R. 2001. Pitavastatin enhanced BMP-2 and osteocalcin expression by inhibition of Rho-associated kinase in human osteoblasts. Biochem Biophys Res Commun 287:337–342.

Osyczka AM, Diefenderfer DL, Bhargave G, Leboy PS. 2004. Different effects of BMP-2 on marrow stromal cells from human and rat bone. Cells Tissues Organs 176: 109–119.

Pan HY, DeVault AR, Wang-Iverson D, Ivashkiv E, Swanson BN, Sugerman A. 1990. A comparative pharmacokinetics and pharmacodynamics of pravastatin and lovastatin. J Clin Pharmacol 30:1128–1135.

Phillips BW, Belmonte N, Vernochet C, Ailhaud G, Dani C. 2001. Compactin enhances osteogenesis in murine embryonic stem cells. Biochem Biophys Res Commun 284: 478–484.

Pisano JM, Colon-Hastings F, Birren SJ. 2000. Postmigratory enteric and sympathetic neural precursors share common, developmentally regulated, responses to BMP2. Dev Biol 227:1–11.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. 1999. Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147.

Quion JA, Jones PH. 1994. Clinical pharmacokinetics of pravastatin. Clin Pharmacokinet 27:94–103.

Sakou T. 1998. Bone morphogenetic proteins: From basic studies to clinical approaches. Bone 22:591–603.

Song S, Guo Z, Ma Q, Chen Z, Liu Z, Jia H, Dang G. 2003. Simvastatin induces osteoblastic differentiation and inhibits adipocyte differentiation in mouse bone marrow stromal cells. Biochem Biophys Res Commun 308:458–462.

Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S. 2000. Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun 271:688–692.

Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW. 1995. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 332:481–487.

Veillard NR, Mach F. 2002. Statins: The new aspirin? Cell Mol Life Sci 59:1771–1786.

Vita JA, Yeung AC, Winniford M, Hodgson JM, Treasure CB, Klein JL, Werns S, Kern M, Plotkin D, Shih WJ, Mitchel Y, Ganz P. 2000. Effect of cholesterol-lowering therapy on coronary endothelial vasomotor function in patients with coronary artery disease. Circulation 102:846–851.

Walter DH, Schachinger V, Elsner M, Mach S, Auch-Schwelk W, Zeiher AM. 2000. Effect of statin therapy on restenosis after coronary stent implantation. Am J Cardiol 85:962–968.

Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T, Nishimura H, Losordo DW, Asahara T, Isner JM. 2002. Statin therapy accelerates reendothelialization: A novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation 105:3017–3024.

Yung SY, Gokhan S, Jurcsak J, Molero AE, Abrajano JJ, Mehler MF. 2002. Differential modulation of BMP signaling promotes the elaboration of cerebral cortical GABAergic neurons or oligodendrocytes from a common sonic hedgehog-responsive ventral forebrain progenitor species. Proc Natl Acad Sci USA 99:16273–16278.