Gene expression profile in osteoblastic differentiation of bone marrow stromal cells stimulated by simvastatin

*Gene chip analysis**★

Meng Ya-qiang[1], Zhang Liu[1], Tian Fa-ming[1,2], Han Da-cheng[1], Zheng Jie[1], Cai Jun[1]

Abstract

BACKGROUND: Simvastatin enhanced the expression of bone morphogenetic protein-2 (BMP-2), which plays an anabolic role in bone metabolism and osteoblastic lineage differentiation. However, little is known about the molecular mechanism of simvastatin on regulation of bone marrow stromal cells differentiation.

OBJECTIVE: To investigated the effect of simvastatin on osteoblastic differentiation of bone marrow stromal cells based on genetics level.

METHODS: Bone marrow stromal cells derived from femur and tibia were cultured in different mediums with simvastatin or vehicle for 7 days. Following extraction and purification, mRNA was reverse-transcripted into cDNA. Fluorescence labeling was employed and the samples were then hybridized with oligonucleotide chip to screen the different genes, which were utilized to analyze osteogenesis-related factors. Alkaline phosphatase and Von Kossa staining were performed at days 14 and 21, respectively.

RESULTS AND CONCLUSIONS: At day 14, alkaline phosphatase-positive cells were more in the experimental group than control group. Von Kossa staining demonstrated that simvastatin could promote BMSCs osteoblastic differentiation and mineralization. Comparative analysis showed that 103 genes out of 22 575 rat genes had differential expression (≥ 2 fold or ≤ 0.5 fold), and some genes were related to cell proliferation and ostoeblastic differentiation, including C/EBP δ , Cited, Ascl2, Ptpn16, Wisp2, Tieg, *etc*. Simvastatin could induce osteoblastic differentiation of bone marrow stromal cells, involving in many osteogenetic-related genes.

[1]Department of Orthopaedic Surgery, Affiliated Hospital of North China Coal Medical University, Tangshan 063000, Hebei Province, China; [2]Hebei Medical University, Shijiazhuang 050017, Hebei Province, China

Meng Ya-qiang★, Master, Physician, Department of Orthopaedic Surgery, Affiliated Hospital of North China Coal Medical University, Tangshan 063000, Hebei Province, China

Correspondence to: Zhang Liu, Doctor, Professor, Doctoral supervisor, Department of Orthopaedic Surgery, Affiliated Hospital of North China Coal Medical University, Tangshan 063000, Hebei Province, China
zhliu130@sohu.com

Supported by: the Natural Science Foundation of Hebei Province, No.C2006000580*

Received: 2009-08-09
Accepted: 2009-11-26
(20090809001/W•H)

Meng YQ, Zhang L , Tian FM, Han DC, Zheng J, Cai J. Gene expression profile in osteoblastic differentiation of bone marrow stromal cells stimulated by simvastatin: Gene chip analysis Zhongguo Zuzhi Gongcheng Yanjiu yu Linchuang Kangfu. 2010;14(11): 2080-2085.

[http://www.crter.cn http://en.zglckf.com]

INTRODUCTION

Mundy *et al*[1] firstly reported that statins enhanced the expression of bone morphogenetic protein-2 (BMP-2) mRNA, which was one of the most examined and potent growth factors targeting bone formation. Subsequently, there was considerable evidence from both *in vivo* and *in vitro* animal studies as well as experimental models in cells lines supporting that statins were a group of potent stimulator of new bone formation[2-3]. All of these independent studies provided a plausible links between the anabolic effect of statins on bone metabolism and osteoblastic lineage differentiation. However, little was known about the effect of statins on the regulation of osteoblast function. Oligonucleotides microarray chip is a molecular technique that enables the analysis of gene expression prolife in parallel on a very large number of genes, the overall result is called genetic portrai[4]. The result also corresponds to up- and down-regulated genes in the investigated cell system. Microarray technology has been used to answer several questions in osteoblast biology. In contrast, there have been few reports on the gene expression changes in osteoblasts cultured with statins using microarray.

In the present study, we defined the effect of simvastatin on the gene expression prolife in rat bone marrow stromal cells (BMSCs) using microarray slides containing 22 575 different oligonucleotides.

MATERIALS AND METHODS

Design

Single sample observation.

Time and setting

This experiment was performed at the Central Laboratory, North China Coal Medical University between September 2006 and March 2007.

Materials

DMEM (Gibco, USA), bovine serum (Haoyang Co., Tianjin, China), simvastatin (Merck), β-glycerophosphate sodium (Sigma), Trizol (Invitrogen), low RNA input liner application and labeling kit plus, stabilization and drying solution (Agilent), Rneasy Mini kit (Qiagen), Cy5NTP, and Cy3NTP (PerkinElmer) were employed in this study. Rat oligonucleotides microarray (G4130A) was purchased from Shanghai Biochip Co., Ltd. Six-week old Sprague Dawley rats weighing 150 g-180 g were provided by Vital River, Beijing, China. The entire experimental procedure was in accordance with the *Guidance Suggestion for the Care and Use of Laboratory Animals*, the Ministry of Science and Technology of the People's Republic of China[5].

Methods

Cell culture

Bone Marrow stromal cells were obtained from six-week old Sprague Dawley rats. BMSCs were repeatedly flushed from the tibias and femurs with culture medium according to the protocol of Ishaug[6]. Bone marrow cells were collected and centrifuged at 800-1 000 rpm for 10 minutes. The resulting cell pellet was resuspended in basal medium consisting of alpha-minimum essential medium (HyClone, USA), 10% fetal bovine serum (Haoyang Co., Tianjin, China), 1% penicillin/streptomycin (Sigma Chemical

Co.,USA), 50 μg/mL ascorbic acid (AA) and 10 mmolβ-glycerophosphate sodium (GP) (Sigma). Cells were seeded in cell culture plastics (Corning Costar, USA) and maintained in 5% CO_2 humidified atmosphere at 37 ℃. Three days after plating, cells were cultured for 4-21 days in osteoblastic differentiation-induced medium. Cells were exposed to simvastatin at a final concentration of 1×10^{-7} mol/L (Merck, Germany; dissolved in 75% ethanol) to be treatment group (group SIM), medium for control group contained equal concentration vehicle (ethanol) (group V). Medium was replaced every 2-3 days, red blood cells and non-adherent cells were removed after first replacement.

Alkaline phosphatase (ALP) and Von Kossa staining

For ALP staining, 14 days after primary culture, cells in 35 mm culture wells were washed twice with phosphate buffer saline (PBS), fixed with 1 mL/well formalin/methanol/H2O (1:1:1.5) for 15 minutes at room temperature, and washed three times with water. 2 mL naphthol-AS-MX phosphate solution and fast red violet B (Blood laboratory, Co. Tianjin, China) was added to the fixed cultures for 2 hours at 37 ℃. After staining, cultures were washed three times with water and air-dried. For Von Kossa staining, 21 days after primary culture, cells were rinsed twice in phosphate buffer saline, fixed with 4% paraformaldehyde for for 5 minutes, 5% silver nitrate were added, being to ultraviolet lamp for an hour until the calcium turns black, and then were added 5% sodium thiosulfate to neutralize the residual silver nitrate, air-dried and preserved at 4 ℃ at last.

RNA extraction

Seven days after primary culture, total RNA in the adherent culture cells were extracted with TRIzol (Invitrogen, USA). After the RNA extraction, RNA was cleaned up using RNeasy Mini Kit (Qiagen, USA). Moreover, DNase Ⅰ (Qiagen) digestion on the column was performed to avoid genomic DNA contamination. The concentration of RNA was quantified by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer, and the purity of RNA was accessed by the ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}), and moreover, the integrity and size distribution of RNA was checked by formaldehyde-agarose gel electrophoresis.

Olige microarray screening and analysis

RNA samples of both groups were studied using oligonucleotides microarray analysis. Double-stranded cDNA synthesis, biotin-labeled cRNA synthesis, and cRNA fragmentation were all conducted as described in the Agilent's Low RNA Input Fluorescent Linear Amplification kit (Agilent Co., USA) protocol. Probes from treatment group were labeled with cyanine 3-CTP and those from control group with cyanine 5-CTP. The fluorescein-labeled cRNA probes were purified and quantified. Agilent's unique rat oligo microarray (G4130A) was comprised of 22 575 (60 mer-) oligonucleotides probes, reprensenting over 20 000 well characterized rat genes, ESTs and EST clusters. After labeling, the oligo microarrays were hybridized with 1 μg of fragmented, biotin-labeled cRNA at 60 ℃ for 16 hours, and the chips were washed at 25 ℃ and stored in the dryer avoiding light. The chips were scanned with the agilent scanner (Agilent Co., USA) and the scope of crossover points was determined, obtaining intensity of the fluorescent signal. Finally, feature extraction software 9.1.3 (Agilent) was used to normalize treatment and analysis. The ratio value of the treatment group/control group was cy3/cy5. The standard value to screen out differently expressed genes was the ratio ≥2.0 or ≤0.5.

Main outcome measures

Alkaline phosphatase analysis at 14 days and Von Kossa staining at 21 days were performed to assay the differentiation of osteoblast. By using oligonucleotides microarray chip containing 22 575 rat genes, we examined the osteoblast-related gene expression profile that leads to ostoeblast lineage from bone marrow stromal cells.

Statistical analysis

The SPSS 10.0 statistical analysis software was used for statistical analysis. Paired-sample T test was used for the comparison of ALP staining assay. *P* value < 0.05 was considered statistically significant.

RESULTS

Total RNA extraction and mRNA purification (Figure 1).

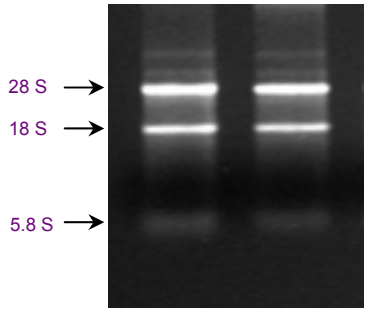


Figure 1 Agarose gel electrophoresis for RNA analysis

The A_{260}/ A_{280} value of total RNA extracted from the treatment group and the control group ranged from 2.11 to 2.15. Analyzing of RNA using 1.0 % agarose gel electrophoresis showed that 18 S rRNA and 28 S rRNA straps were clear, suggested that we have acquired the highly purified total mRNA.

Result of the gene microarray hybridization

After microarray hybridizations, significant changes in gene expression were found in totally 103 genes. Figure 2 represents the hybridization signal intensity scatter plot. Among them, 35 genes were up-regulated and 68 genes were down-regulated. We searched for the database of these genes with the BLASTn and BLASTx sequence comparison programs of the National Center for Biotechnology Information (NCBI) and found the up-regulated and down-regulated genes which responsible for osteoblast differentiation from BMSCs.

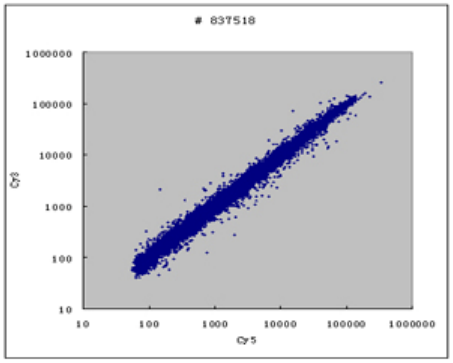


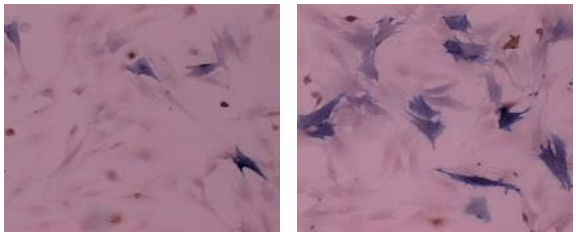
Figure 2 Signal intensity scatter plot for microarray hybridization

Microarray analysis data of differentially expressed bone-related genes are as follows:

Genebank ID	Gene Name	Symbol	Radio (Cy5/Cy3)	Gene functional classification
NM_013154	CCAAT/enhancer binding protein delta	C/EBPδ	4.675477↑	Regulation of transcription
NM_053698	Cbp/p300-interacting transactivator	Cited	22.047966↑	Regulation of transcription
NM_031503	Achaete-scute homolog 2, mRNA	Ascl2	0.437809↓	Regulation of transcription
NM_053769	Protetyrosine phosphatase, non-receptor type 16	Ptpn16	3.018164↑	Erzymes
NM_031135	TGFβ inducible early growth response	Tieg	0.271637↓	CF*/GF△
NM_031590	WNT1 inducible signaling pathway protein 2	Wisp2	0.431696↓	Signal conduction
NM_053847	mitogen-activated protein kinase kinase kinase 8	Map3k8	0.3126↓	Signal conduction
NM_139089	Chemokine (C-X-C motif) ligand 10	Cxcl10	0.1447↓	CF/GF
NM_017019	Interleukin 1 alpha	IL1a	0.4664↓	CF/GF
NM_012854	Interleukin 10	IL 10	0.339↓	CF/GF

* cell factor; △ growth factor

Effect of simvastatin on (ALP) expression (Figure 3)

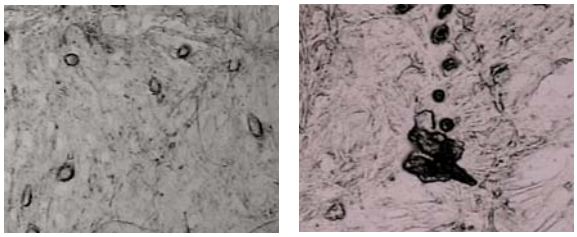


V SIM

Figure 3 Staining of alkaline phosphatase at day 14 (×100)

We examined the expression of ALP, an enzyme serving as a marker of osteoblast differentiation in culture of rat bone marrow stromal cells. Simvastatin increased the ALP expression 14 days after culture, indicated by a higher positive cell (dark blue) rate compared to that of group V (SIM: 0.36±0.14; V: 0.14±0.11) ($P < 0.05$).

Effect of simvastatin on mineralized nodule formation (Figure 4)



V SIM

Figure 4 Staining for mineralized bone nodules with Von Kossa staining (×200)

At the day 21, we tested the effect of simvastatin on osteoblast differentiation by observing mineralization, simvastatin showed positive effect on bone mineralization of BMSCs.

DISCUSSION

The bone marrow stromal contains multipotent cells that are capable of differentiating into osteoblasts, chondrocytes, adipocytes, and myoblasts. BMSCs have the characteristics of self-proliferation and multi-differentiation[7]. During osteogenesis, different cellular phenotypes were tentatively defined as osteoprogenitors, preosteoblasts, mature osteoblasts and mineralizing osteoblasts, each of them characterized by an overlapping set of marker genes[8]. Classical cytochemical markers of osteoblast differentiation are alkaline phosphatase and mineralized bone nodules. This study showed that simvastatin, a HMG-CoA reductase inhibitor, promoted osteoblastic differentiation in rat BMSCs. Simvastatin stimulated the ALP activity, which is an early osteoblastic differentiation marker and promoted the mineralization of the matrix by osteoblasts. These results are consistent with previous observations showing that the statins enhanced osteogenesis in MC3T3-E1 cells (a clonal pre-osteoblastic cell line derived from mouse calvaria) and aging rat marrow stromal cells[9-10]. Previously, the effect of simvastatin on osteoblast differentiation was examined mostly at cellular or single gene level. Many external regulating factors, critical molecular steps in osteoblast differentiation and bone formation are largely unknown. The rat oligonucleotide array used in the present study has enabled us to specifically examine a large number of genes involved in the process of osteoblast differentiation. In our study, we examined the expression profiles of 22575 genes of BMSCs, 103 genes provided significant signals (Ratio Cy3/Cy5 ≥2 or ≤0.5). Among them more than 10 genes are related to ostoeblastic lineage differentiation.

The upregulated genes involved in transcription factor are C/EBPδ, Cited2. CCAAT-enhancer binding proteins (C/EBP) are a family of transcription factors that regulate cell differentiation[11]. C/EBPs are expressed in multiple cell types including osteoblasts and adipocytes, and play critical roles in adipocyte differentiation. C/EBPδ regulates IGF I transcription, and activates osteocalcin

transcription by interacting with runt related protein (Runx)-2[12-13]. The Runx2 transcriptional activators are critical factors for the development of hematopoietic and skeletal tissues. Early during the process of differentiation, Cbp/p300-interacting transactivator 2 (Cited2) is upregulated. Cited2 is a transcriptional co-activator that physically sociates with Cbp/p300 enhancing its ability to transactivate a variety of genes[14]. Cbp/p300 activates gene expression by acting as a bridge between activators and the general transcriptional machinery and may regulate transcription by enhancing chromatin remodeling.

We found that a number of genes involved in some well known osteogenesis related signaling pathways such as TGFβ/BMP pathway[15-16], mitogen-activated protein kinase (MAPK) pathway[17-19], WNT/β-catenin pathway were up-regulated or down-regulated[20-23]. For example, two of the critical enzymes that participate in MAPK signal pathway (Ptpn16, Map3k8) were differentially expressed. Xiao *et al*[24] found that the MAPK pathway provides a plausible link between cell surface integrin activation by ECM and subsequent stimulation of Cbfa1-dependent transcription, suggesting that this pathway has an important role in the control of osteoblast-specific gene expression. Some proteins involved in WNT/β-catenin pathway (Wisp2) were also down-regulated. Clearly, how these regulatory signals affect osteoblast activity are needed to further study. Cytokines, such as Cxcl10, Il10, Il1a, showed differential expression after simvastatin stimulation for 4 days. Lisignoli *et al*[25] demonstrated that stimulation of OBs with CXCL10 significantly induces both b-N-acetylhexosaminidase (Hex) release and ALP activity. Hex is an enzyme used to evaluate the standard response of leukocytes after chemokine stimulation, but it is also known to play a role in endochondral ossification and bone remodeling[26-27], since it degrades glycosaminoglycans. As reported by other authors, CXCL10 is highly effective in the inhibition of primary human microvascular endothelial cell proliferation and in stimulating human mesangial cells[28-29]. These different effects of CXCL10 on the proliferation of various cell types may be due to the phenotypical characteristics of cells or to the level of cell differentiation. In another study, they also found that CXCL10 and CXCL13 induced a dose-dependent increase of cell proliferation in OB from young donors[30].

In conclusion, in this study we have identified the multiple biological events as reflected by gene expression prolifes in the early period of ostoeblastic lineage differentiation of bone marrow stromal cells treated with simvastatin, as well as the promoting effects of simvastatin on BMSCs differentiation and minerlization indicating by ALP staining and Von Kossa Staining. Simvastatin is able to modulate a broad range of biological processes. The precise interaction between these genes is not clear. Clearly, more studies are needed. First, primary cell culture may contain heterogenous cell population, often containing contaminated cells of different types and cells in variable differentiation states. This may have leaded to a less precise demonstration of the effect of simvastatin on BMSCs. However, we believe that the present report provides new data on the genetic effect of simvastatin on bone marrow stromal cells.

REFERENCES

[1] Mundy G, Garrett R, Harris S, et al. Stimulation of bone formation in vitro and in rodents by statins. Science. 1999;286:1946-1949.

[2] Pauly S, Luttosch F, Morawski M, et al. Simvastatin locally applied from a biodegradable coating of osteosynthetic implants improves fracture healing comparable to BMP-2 application. Bone. 2009;45(3):505-511.

[3] Ho ML, Chen YH, Liao HJ, et al. Simvastatin increases osteoblasts and osteogenic proteins in ovariectomized rats. Eur J Clin Invest. 2009;39(4):296-303.

[4] Carinci F, Francioso F, Rubini C. Genetic portrait of malignant granular cellodontogenic tumour. Oral Oncology. 2003;39(1): 69-77.

[5] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.

[6] Ishaug SL, Crane GM, Miller MJ, et al. Bone formation by three-dimensional stromal osteoblast culture in biodegradable polymer scaffolds. J Biomed Mater Res. 1997;36(1):17-28.

[7] Vayssade M, Nagel MD. Stromal cells. Front Biosci. 2009; 14:210-224.

[8] Zou L, Zou X, Chen L, et al. Multilineage differentiation of porcine bone marrow stromal cells associated with specific gene expression pattern. J Orthop Res. 2008;26(1):56-64.

[9] Maeda T, Matsunuma A, Kawane T, et al. Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. Biochem Biophys Res Commun. 2001;280(3):874-877.

[10] Liu M, Wang K, Tang T, et al. The effect of simvastatin on the differentiation of marrow stromal cells from aging rats. Pharmazie. 2009;64(1):43-48.

[11] Ramji DP, Foka P. CCAAT/enhancer-binding proteins: structure, function, and regulation. Biochem J. 2002;365(Pt 3):561-575.

[12] Gutierrez S, Javed A, Tennant DK, et al. CCAAT/enhancer-binding proteins (C/EBP) β and δ activate osteocalcin gene transcription and synergizewith runx2 at the C/EBP element to regulate bone-specific expression. J Biol Chem. 2002;277(2): 1316-1323.

[13] Delany AM, Durant D, Canalis E. Glucocorticoid suppression of IGF I transcription in osteoblasts. Mol Endocrinol. 2001;15(10): 1781-1789.

[14] Braganca J, Eloranta JJ, Bamforth SD, et al. Physical and functional interactions between AP-2 transcription factors, p300/CBP, and CITED2.J Biol Chem. 2003;278(18):16021-16029.

[15] Hauburger A, von Einem S, Schwaerzer GK, et al. The pro-form of BMP-2 interferes with BMP-2 signalling by competing with BMP-2 for IA receptor binding. FEBS J. 2009;276(21):6386-6398.

[16] Shockley KR, Lazarenko OP, Czernik PJ, et al. PPARgamma2 nuclear receptor controls multiple regulatory pathways of osteoblast differentiation from marrow mesenchymal stem cells. J Cell Biochem. 2009;106(2):232-246.

[17] Wang Y, Cui K, Zhao H, et al. Bushen Ningxin Decoction pharmacological serum promotes the proliferation and suppresses the apoptosis of murine osteoblasts through MAPK pathway. J Ethnopharmacol. 2009;122(2):221-226.

[18] Peng S, Zhou G, Luk KD, et al. Strontium promotes osteogenic differentiation of mesenchymal stem cells through the Ras/ MAPK signaling pathway. Cell Physiol Biochem. 2009; 23(1-3):165-174.

[19] Franceschi RT, Ge C, Xiao G, et al. Transcriptional regulation of osteoblasts. Cells Tissues Organs. 2009;189(1-4):144-152.

[20] Yavropoulou MP, Yovos JG. The role of the Wnt signaling pathway in osteoblast commitment and differentiation. Hormones (Athens). 2007;6(4):279-294.

[21] Qiang YW, Hu B, Chen Y, et al. Bortezomib induces osteoblast differentiation via Wnt-independent activation of beta-catenin/TCF signaling. Blood. 2009;113(18):4319-4330.

[22] Hartmann C. Skeletal development-Wnts are in control.Mol Cells. 2007;24(2):177-184.

[23] Deng ZL, Sharff KA, Tang N, et al. Regulation of osteogenic differentiation during skeletal development. Front Biosci. 2008; 13:2001-2021.

[24] Xiao G, Jiang D, Thomas P, et al. MAPK Pathways activate and phosphorylate the osteoblast-specific transcription factor, Cbfa1. J Biol Chem. 2000;275(6):4453-4459.

[25] Lisignoli G, Toneguzzi S, Piacentini A, et al. Human osteoblasts express functional CXC chemokine receptors 3 and 5: activation by their ligands, CXCL10 and CXCL13, significantly induces alkaline phosphatase and beta-N- acetylhexosaminidase release. J Cell Physiol. 2003; 194(1): 71-79.

[26] Gamble JG, Haimson R, Smith RL. Glucosaminidase, galactosaminidase, and glucuronidase in the growth plate. J Orthop Res.1990;8(5):764-768.

[27] Bodo M, Carinci P, Venti G, et al. Glycosaminoglycan metabolism and cytokine release in normal and otosclerotic human bone cells interleukin-1 treated. Connect Tissue Res. 1997;36(3):231-240.

[28] Romagnani P, Beltrame C, Annunziato F, et al. Role for interactions between IP-10/Mig and CXCR3 in proliferative glomerulonephritis. J Am Soc Nephrol. 1999;10(12):2518-2526.
[29] Romagnani P, Annunziato F, Lasagni L, et al. Cell cycledepedent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. J Clin Invest. 2001;107(1):53-63.
[30] Lisignoli G, Piacentini A, Toneguzzi S, et al. Age-associated changes in functional response to CXCR3 and CXCR5 chemokine receptors in human osteoblasts. Biogerontology. 2003;4(5):309-317.

辛伐他汀作用下大鼠骨髓基质干细胞向成骨细胞分化中相关基因表达谱：基因芯片的分析*★

孟亚强[1]，张　柳[1]，田发明[1,2]，韩大成[1]，郑　杰[1]，蔡　俊[1]([1]华北煤炭医学院附属医院骨外科，河北省唐山市　063000；[2]河北医科大学，河北省石家庄市　050017)

孟亚强★，男，1979 年生，山西省介休市人，汉族，2004 年华北煤炭医学院毕业，硕士，医师，主要从事骨质疏松的临床与基础研究。
通讯作者：张柳，博士，教授，博士生导师，华北煤炭医学院附属医院骨外科，河北省唐山市　063000

摘要

背景：辛伐他汀可上调骨形态发生蛋白 2 的表达从而促进骨形成，但是具体分子水平的作用机制尚不清楚。

目的：从基因水平明确辛伐他汀在体外诱导大鼠骨髓基质干细胞向成骨细胞分化过程中对成骨相关基因表达谱的影响。

方法：取大鼠股骨、胫骨骨髓基质干细胞进行体外培养，给予辛伐他汀或安慰剂干预，培养第 7 天，提取、纯化 mRNA，反转录合成 cDNA，荧光标记后与大鼠全基因组寡核苷酸芯片杂交、扫描后筛选出差异表达的基因，分析与成骨分化相关基因。第 14，21 天分别进行碱性磷酸酶和钙结节茜素红染色。

结果与结论：第 14 天，碱性磷酸酶染色阳性细胞比例实验组明显多于对照组；茜素红染色表明辛伐他汀能促进成骨细胞的矿化能力。在 22 575 个基因中，共检出 2 倍差异表达基因和表达序列标记(ESTs)103 条，其中包括与细胞增殖及成骨分化相关差异表达基因，如 C/EBP δ，Cited，Ascl2，Ptpn16，Wisp2，Tieg 等。结果表明，辛伐他汀能够促进骨髓基质干细胞向成骨细胞分化，其作用机制与从基因水平调控多种成骨相关基因的表达有关。

关键词：辛代他汀；骨髓基质干细胞；成骨细胞；基因；大鼠；骨组织工程

doi:10.3969/j.issn.1673-8225.2010.11.045
中图分类号: R318　文献标识码: A
文章编号: 1673-8225(2010)11-02081-05
孟亚强，张柳，田发明，韩大成，郑杰，蔡俊. 辛伐他汀作用下大鼠骨髓基质干细胞向成骨细胞分化中相关基因表达谱：基因芯片的分析[J].中国组织工程研究与临床康复，2010，14(11):2081-2085.
[http://www.crter.org　http://cn.zglckf.com]
(Edited by Ji H/Wang L)

来自本文课题的更多信息--

基金资助：河北省自然科学基金项目(C2006000580)。

利益冲突：无利益冲突。

Matching categorical object representations in inferior temporal cortex of man and monkey ④

Our approach has the following key features. (1) The same particular images of real-world objects are presented to both species while measuring brain activity in IT (with electrode recording in monkeys and high-resolution fMRI in humans). (2) Stimuli are presented in random sequences; neither the experimental design nor the analysis is biased by any predefined grouping. (3) Each stimulus is treated as a separate condition, for which a response pattern is estimated without spatial smoothing or averaging[5-6]. (4) The analysis targets the information in distributed response patterns[7-8]. (5) In order to compare the IT representations between the species and to computational models, we use the method of "representational similarity analysis" [9], in which RDMs are visualized and quantitatively compared.

Population representations of the same stimuli have not previously been compared between monkey and human. However, our approach is deeply rooted in the similarity analyses of mathematical psychology[10]. An introduction is provided by Edelman[11], who pioneered the application of similarity analysis to fMRI activity patterns[12] using the technique of multidimensional scaling[13-15]. Several studies have applied similarity analyses to brain activity patterns and computational models[16-20].

Beyond the species comparison, our approach allows us to address the question of categoricality. IT is thought to contain a population code of features for the representation of natural images of objects[21-25]. Does IT simply represent the visual appearance of objects? Or are the IT features designed to distinguish categories defined independent of the visual appearance of their members?

Whether IT is optimized for the discrimination of object categories is unresolved. Human neuroimaging has investigated category-average responses for predefined conventional object categories[26-32]. This approach requires the assumption of a particular category structure and therefore cannot address whether the representation is inherently categorical. Monkey studies have reported IT responses that are correlated with categories[33-40]. However, more clearly categorical responses have been found in other regions[41-42], suggesting that IT has a lesser role in categorization[43].

Kiani *et al*[44] investigated monkey-IT response patterns elicited by over 1 000 images of real-world objects to address whether IT is inherently categorical. The present study uses the same monkey data and a subset of the stimuli to compare the species. Cluster analysis of the monkey data revealed a detailed hierarchy of natural categories inherent to the monkey-IT representation.

Will human-IT show a similar categorical structure? Our approach allows us to address the question of categoricality without the bias of predefined categories. Independent of the result, this provides a crucial piece of evidence for current theory. The question of the inherent category structure of IT is of particular interest with respect to the species comparison, because the prevalent categorical distinctions might be expected to differ between species.

Our goal is to investigate to what extent monkey and human-IT represent the same object information. In particular, we ask the following. (1) Do human-IT response patterns form category clusters as reported for monkey IT[45]? If so, what is the categorical structure and does it match between species? (2) Is within-category exemplar information present in IT? If so, is this continuous information consistent between the species? (3) How is the representation of the objects transformed between early visual cortex and IT? (4) What computational models can account for the IT representation?