pcDNA$_{3.1}$-osteogenic growth polypeptide eukaryotic expression vector in bone marrow mesenchymal stem cells***★

An Gang, Lü Song-cen, Guo Ya-shan, Xue Zhen, Deng Qiu-kui

Department of Orthopaedics, Second Affiliated Hospital, Harbin Medical University, Harbin 150086, Heilongjiang Province, China

An Gang★, Master, Physician, Department of Orthopaedics, Second Affiliated Hospital, Harbin Medical University, Harbin 150086, Heilongjiang Province, China abc7231719@163.com

Guo Ya-shan★, Master, Attending physician, Department of Orthopaedics, Second Affiliated Hospital, Harbin Medical University, Harbin 150086, Heilongjiang Province, China guoyashan1972@163.com

An Gang and Guo Ya-shan contributed equally to this study.

Correspondence to: Lü Song-cen, Doctor, Professor, Department of Orthopaedics, Second Affiliated Hospital, Harbin Medical University, Harbin 150086, Heilongjiang Province, China lv226918@163.com

Supported by: the Returned Students Fund of Heilongjiang Province, No. LC04C03*; the Youth Scientific Research Fund of Harbin City, Training Reserve Leader of Subject Fund, the Returned Students Fund Project, No. 2005AFLXJ016*

Received: 2011-03-19
Accepted: 2011-05-30
(20110125003/WLM)

Abstract

BACKGROUND: Osteogenic growth polypeptide (OGP) had clear effect on promoting osteoblast proliferation, differentiation and mature.

OBJECTIVE: To explore the expression of OGP gene, which was transfected into rabbit bone marrow mesenchymal stem cells (BMSCs) and to evaluate the effects of OGP on differentiation of rabbit BMSCs.

METHODS: pcDNA$_{3.1}$-OGP was constructed using gene cloning and recombination techniques. Rabbit BMSCs were transfected with pcDNA$_{3.1}$-OGP mediated by lipofectamine 2000. The transfection positive cell clones were selected with G418. The expression of OGP gene was detected using reverse transcription-polymerase chain reaction analysis on an mRNA level. Differentiation of pcDNA$_{3.1}$-OGP transfected BMSCs into osteoblast lineage was observed.

RESULTS AND CONCLUSION: The pcDNA3.1-OGP plasmid was constructed successful and OGP expression was detected in rabbit BMSCs. Hydroxyproline content was increased, and alkaline phosphatase activity was also increased. These indicate that pcDNA3.1-OGP transfected BMSCs expressed OGP, and could differentiate into osteoblast lineage.

INTRODUCTION

Bone marrow mesenchymal stem cells (BMSCs) are widely used progenitor cells in bone tissue engineering studies[1]. Osteogenic growth polypeptide (OGP) is one of the osteogenic polypeptides which promote proliferation of osteoblasts *in vitro* and *in vivo*. OGP was fist isolated by Bab *et al*[2-3] from healing bone marrow cells. Its molecular formula is H2N-ALKRQGRTLYGFGG-COOH[4] and its molecular weight is 1 523. In physiological condition, 80%–97% of OGP exists in the OGP-OGPBP compound in human and mammalian serum[2-3]. OGP was proved to promote osteoblast proliferation, differentiation and sophistication *in vitro*, and increase whole body bone quantity *in vivo*[5]. As the development of gene therapy and tissue engineering technology, it becomes an ideal method for bone regeneration and bone defect restoration to introduce bone induction factor genes into BMSCs and expect local OGP expression to exhibit bone tissue induction activity. In this study, synthetic OGP was recombinated into the eukaryotic expression vector as the pcDNA$_{3.1}$-OGP plasmid, which was subsequently transfected into rabbit BMSCs. Expression of OGP in BMSCs could lay the foundation of gene therapy for bone repair and regeneration.

MATERIALS AND METHODS

Design

Cytological level, contrast observational study.

Time and setting

Experiments were performed at the Harbin Medical University in China in July 2006.

Materials

One healthy 4–8 weeks-old male New Zealand rabbit, weighing 2.0 kg, was provided by the Laboratory Animal Center, Harbin Medical University. Experimental protocol was in accordance with animal ethical standard.

Reagent and instrument:

Reagent and instrument	Source
pcDNA3.1 eukaryotic expression vector, liposome Lipofectamine^{TM} 2000 tranfection kit, G418	Invitrogen, USA
Competent E coli JM 109, restriction enzyme, T4 DNA ligase	Takara, USA
Alkaline phosphatase (ALP) assay kit, hydroxyprolineassay kit	Nanjing Jiancheng, China
Polymerase chain reaction (PCR) technology and gene amplification analysis instrument	Bio-Rad, USA

Methods

Selections of target gene and eukaryotic expression vector

Synthetic OGP gene was provided by the Biochemical Department of Harbin Medical University. The sequences of target gene were as follows: sense: 5'-AGCT ATG GCG CTT AAA CGC CAG GGC CGC ACA CTC TAC GGC TTC GGT GGT TAA-3'; antisense: 5'-TCGA TTA ACC ACC GAA GCC GTA GAG TGT GCG GCC CTG GCG TTT AAG CGC CAT-3'. pcDNA$_{3.1}$ was used as eukaryotic expression vector. Target gene was ligated into Hind Ⅲ and *Xho* Ⅰ double digestion sites, which were two of the more distant sites in pcDNA$_{3.1}$ vector.

Purification of the eukaryotic expression vector pcDNA$_{3.1}$ and recycling of target gene fragment

Eukaryotic expression vector pcDNA$_{3.1}$ was digested with *Xho* Ⅰ and *Hin*d Ⅲ restriction enzymes at 37 ℃ for 6 hours. The samples were electrophoresed in

An G, Lü SC, Guo YS, Xue Z, Deng QK. pcDNA$_{3.1}$-osteogenic growth polypeptide eukaryotic expression vector in bone marrow mesenchymal stem cells. Zhongguo Zuzhi Gongcheng Yanjiu yu Linchuang Kangfu. 2011;15(36): 6696-6700.

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

0.8% agarose gel for 30 minutes, stained with bromophenol blue and DL2000 marker, and then illuminated with ultraviolet light to check if the digestion was complete. Electrophoretic bands were instantly cut and recycled with DNA Purification Recycling Kit for digested eukaryotic expression vector pcDNA$_{3.1}$.

Construction and identification of pcDNA$_{3.1}$-OGP plasmid

After annealing, phosphorylated target gene was incubated with purified eukaryotic expression vector pcDNA and T4DNA joinase at 16 ℃ for 12 hours. JM109 bacteria were coated on LB agar plate containing 100 mg/L of ampicillin and 20 g/L of X-gal. After evaporated at room temperature for 30 minutes, the plate was placed invertedly in the incubator for 12 hours before bacteria colonies appeared. Randomly picked colonies were amplified by shaking. pcDNA$_{3.1}$-OGP plasmid was extracted using plasmid extraction kit. There were single restriction site losses between *Hin*d Ⅲ and *Xho* Ⅰ sites which could not be digested by relevant enzymes. Therefore, BamHI was used to test the purification of pcDNA$_{3.1}$-OGP plasmid. pcDNA$_{3.1}$-OGP plasmid was single digested with BamHI and the results were observed using agarose gel electrophoresis and then sent to Shanghai Biology Engineering for sequencing.

Isolation and culture of rabbit BMSCs

Rabbit BMSCs were isolated and amplified using the density gradient centrifugation technique. The rabbits were injected with sodium pentobarbital (30 mg/kg) intravenously for anesthesia. No. 16 bone marrow puncture needle was connected with 10 mL syringe which contained 2 mL of heparin sodium (2 500 U/mL). In aseptic conditions, lateral tibial tubercle was punctured and 2.0–3.0 mL of bone marrow fluid was collected. Cell suspension was then slowly adherently injected into equal volume of Percoll solution (Density=1.082). A clear interface was formed between the cell suspension and Percoll solution. After centrifuged at 2 500 r/min for 20 minutes, the white film formed by mononuclear cells in the middle of the mixture was drewn and washed twice with PBS. The mononuclear cell suspension was centrifuged at 1 500 r/min for 10 minutes. Supernatant was discarded and 10 mL of DMEM culture solution with 15% of FBS was added to form single-cell suspension, which was incubated in 50-mL culture bottle at 37 ℃ in humidified atmosphere of 5% CO_2. After 3 days of incubation, half volume of the medium was changed and all the non-adherent cells were discarded. Total volume of the medium was changed every third to fifth days thereafter. Cells were passaged twice and subsequently used at passage three for gene transfer studies.

Liposome mediated pcDNA$_{3.1}$-OGP transfection into BMSCs

When the third generation of BMSCs reached 80% confluence, pcDNA$_{3.1}$-OGP plasmid was transfected into BMSCs using Lipofectamine 2000. 24 hours after transfection, G418 (300 μg/mL) was added in the culture medium of part of the cells for screening. Two weeks later, G418-resistant cell clones were collected and cultured with normal medium. BMSCs were transfected with pcDNA$_{3.1}$-OGP plasmid, vehicle or left un-transfected as a control group.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis of OGP expression of BMSCs

Seventy-two hours after transfection, 2×10^6 of rabbit BMSCs were obtained from pcDNA$_{3.1}$-OGP transfected group, vehicle and control groups respectively. BMSCs of three groups in culture were lysed with TRIzol solution. First strand cDNA was reverse-transcribed from the total RNA with SuperScript^{TM} Ⅱ RNase Reverse Transcriptase. The primer was compounded by Bei-Jing Ying Jun Co.
31ogps9: ACTCTACGGCTTCGGTGGTT
31ogps113: CCACTGCTTACTGGCTTATCG
31ogpa266: GTGAGGGTGACAGGAAAGGA
31ogps9 and 31ogpa266 can augment the cDNA of primitive plasmid and restructuring of plasmids. 31ogps113 and 31ogpa266 only can augment the cDNA of restructuring of plasmids.
PCR amplification was carried out using Invitrogen's PCR Kit. Samples were incubated at 94 ℃ for 4.5 minutes and at 55 ℃ for 30 seconds, and then at 72 ℃ for 30 seconds as one cycle. After 35 cycles of amplification, the amplified products were electrophoresed in 1% agarose gel, stained with 10% ethidium bromide, and the gel was illuminated with ultraviolet light.

Detection of ALP activity

Cells were inoculated into a 24-well plate at a density of 2×10^4/well. Four parallel wells were set for each group. Supernatant was taken at 1, 2, 3, and 4 days to detect ALP activity by ALP assay kit.

Detection of hydroxyproline content by Chloramine-T method

Two weeks later, cells were inoculated into a 24-well plate at a density of 2×10^4/well. Four parallel cells were set in each group. Hydroxyproline content was detected using hydroxyproline assay kit.
Hydroxyproline (mg/L) = Absorbance $_{\text{detected tubes}}$/ Absorbance $_{\text{standard tubes}}$ × concentration of standard tubes × diluted times.

Main outcome measures

OGP mRNA expression, ALP activity and hydroxyproline content were measured in pcD-NA3.1-OGP BMSCs.

Statistical analysis

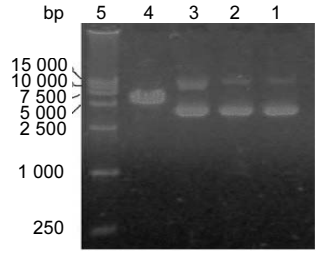
All data were statistically processed by the first author using SPSS 10.0 software and expressed as Mean ± SD. Transfection effects of different

groups were compared using *t* test. The level of significance was set at $P < 0.05$.

RESULTS

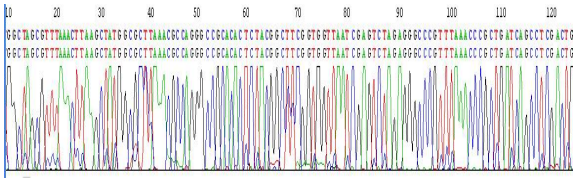
Identification and sequencing of pcDNA$_{3.1}$-OGP plasmid

Plasmid was single digested with BamHI and electrophoresed on agarose gel. PcDNA$_{3.1}$ plasmid was digested into chains with molecular weight of approximately 5.4 kb, while recombined pcDNA$_{3.1}$-OGP plasmid could not be digested and remained in circle shape (Figure 1). The sequencing of the digested products showed a match with the sequence designed (Figure 2), which indicated that eukaryotic expression vector was constructed successfully.



OGP: Osteogenic growth polypeptide; 1: pcDNA$_{3.1}$ plasmid; 2: pcDNA$_{3.1}$-OGP plasmid; 3: Bam HI digested pcDNA$_{3.1}$-OGP plasmid; 4: Bam HI digested pcDNA$_{3.1}$ plasmid; 5: DNA marker

Figure 1 Gel electrophoresis of digested recombinant pcDNA$_{3.1}$-OGP plasmid



OGP: Osteogenic growth polypeptide

Figure 2 pcDNA$_{3.1}$-OGP plasmid sequencing result

BMSCs morphology

Original rabbit BMSCs were round-shaped. 48 hours after inoculation, BMSCs were adherent, stretching in the triangle, polygon and gradually turning into long spindle-shaped shapes. Adherent BMSCs proliferated at day 3 and formed scattered fibroblast colony at day 7. At day 14, colonies integrated and confluenced. Post-transfection BMSCs had similar shapes with polygonal osteoblasts (Figures 3–5).

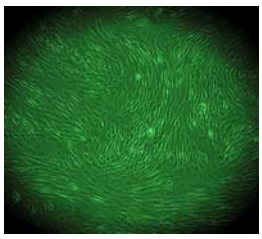
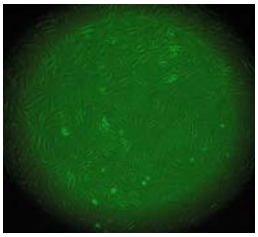


Figure 3 Long spindle-shaped bone marrow mesenchymal stem cells before transfection (Inverted microscope, ×100)

OGP: Osteogenic growth polypeptide; BMSCs: bone marrow mesenchymal stem cells

Figure 4 A small number of pcDNA$_{3.1}$-OGP transfected BMSCs survived 2 hours after G418 screening (Inverted microscope, ×100)

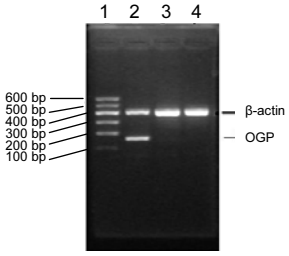


OGP: Osteogenic growth polypeptide; BMSCs: bone marrow mesenchymal stem cells

Figure 5 After G418 screening, pcDNA$_{3.1}$-OGP transfected BMSCs shaped similar with polygonal osteoblasts (Inverted microscope, ×100)

RT-PCR analysis of pcDNA$_{3.1}$-OGP transfected BMSCs

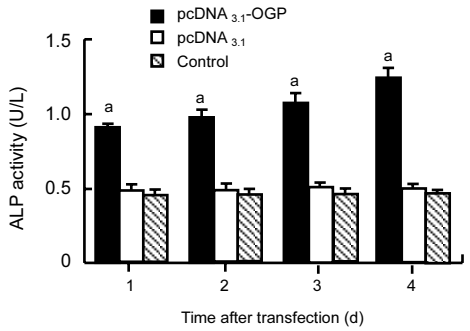
RT-PCR of pcDNA$_{3.1}$-OGP transfected group and pcDNA$_{3.1}$ transfected group both had positive results using primers 31ogps9 and 31ogpa266, and the amplification products were 280 bp and 258 bp respectively. The PCR results of original plasmid were negative using primers 31ogps113 and 31ogpa266, while the amplification products of recombinated plasmid were 154 bp. Results of electrophoresis showed that both original and reconstructed plasmid group had gene transcription. Results of pcDNA$_{3.1}$-OGP transfected group showed specific bands at 154 bp, which indicated that exogenous OGP gene was transfected in rabbit BMSCs at the mRNA level. Results of pcDNA$_{3.1}$ transfected and un-transfected control group were negative (Figure 6).



OGP: Osteogenic growth polypeptide; BMSCs: bone marrow mesenchymal stem cells; 1: DNA marker; 2: pcDNA$_{3.1}$-OGP transfected group; 3: pcDNA$_{3.1}$ transfected group; 4: un-transfected control group

Figure 6 Real-time polymerase chain reaction analysis of pcDNA$_{3.1}$-OGP transfected BMSCs

Detection results of ALP activity (Figure 7)



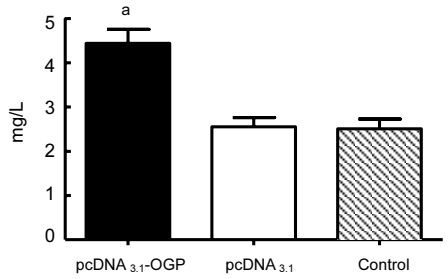
$^aP < 0.05$, *vs.* control group

Figure 7 Effect of osteogenic growth polypeptide (OGP) gene transfection on alkaline phos-phatase (ALP) activity in bone marrow mesenchymal stem cells

ALP activity in the pcDNA$_{3.1}$-OGP transfected group significantly increased at 2 days after transfection, and still increased at 4 days. ALP activity in the pcDNA$_{3.1}$-transfected, and untransfected control group was basically unchanged. At each time point, ALP activity was significantly higher in the pcDNA$_{3.1}$-OGP transfected group than in the pcDNA$_{3.1}$-transfected, and untransfected control group ($P < 0.05$).

Detection of hydroxyproline content by Chloramine-T method

Hydroxyproline content was significantly higher in the pcDNA$_{3.1}$-OGP transfected group than in the pcDNA$_{3.1}$-transfected, and untransfected cells ($P < 0.01$; Figure 8).



OGP: Osteogenic growth polypeptide; $^aP < 0.01$, *vs.* control group

Figure 8 Osteocalcin production in pcDNA$_{3.1}$-OGP transfected group, pcDNA$_{3.1}$-transfected group, and un-transfected control group

DISCUSSION

With the rapid development of tissue and genetic engineering technology, the joint application of bone tissue engineering, which is centered with the combination of progenitor cells and biological materials, and gene modification, which is centered with DNA recombination becomes a hot spot of extensive research[6]. The key of the combination of these two emerging technologies is transferring bone growth factor gene into progenitor cells in bone tissue engineering, so that the proliferation of these cells in the body can promote the secretion of growth factors and bone healing. The problems caused by low implantation effect and short duration can be solved[7]. Bone marrow stromal cells become ideal progenitor cells in bone tissue engineering because of their convenient isolation, small injury when obtained, and strong osteogenic capacity[8]. BMSCs have the potential to differentiate into multiple cells. But their differentiation is not a completely spontaneous process, but can be promoted by a number of cytokines and certain environment *in vivo*. Studies showed that the ossification process was slow using only BMSCs, and transformation was needed for BMSCs to become ideal progenitor cells[9-10]. Therefore, how to enhance the osteogenic potential of BMSCs and secure their transformation to osteoblasts stably and massively are the keys to researches in bone tissue engineering.

OGP is 14-polypeptide growth factor which promotes proliferation of osteoblasts *in vitro* and *in vivo*. Results of domestic and foreign researches demonstrated that OGP had clear promoting effect on osteoblast proliferation, differentiation and sophistication. OGP was used to accelerate fracture healing and prevent osteoporosis because of its capacity of increasing whole body bone mass through affecting ROS, MC3T3, E1 and NH3T3 osteoblast cell lines[11-12]. The mechanism of OGP effects *in vitro* and *in vivo* is still not clear. Brager *et al*[13] observed increasing TGF-β1 expression in the whole body and in the local partial fracture when OGP was used. Expression of 11A, 11B collagen and other factors were also increased, so they considered that OGP had effect on TGF-β system, which contributes to proliferation and differentiation of bone marrow and callus-derived osteoblast cells. OGP is a 14-peptide small molecule. Because of the technical difficulty and high cost, OGP used for research and treatment is all synthetic in domestic and abroad. Nobody has transferred OGP into BMSCs for gene therapy use so far. Direct application of synthesized OGP is highly cost, complex operated, plus the level of drug concentration is hard to maintain stably. Gene therapy can avoid these drawbacks.

In this study, rabbit BMSCs were liposome-mediated transfected with pcDNA3.1-OGP eukaryotic plasmid *in vitro*. 72 hours after transfection, OGP gene transcription in rabbit BMSCs was detected with RT-PCR on the mRNA level. ALP, type Ⅰ collagen are considered as early enzymes of osteoblastic differentiation, and their expression is an important indicator of osteoblast differentiation and function, and a specific indicator of the tissue calcification and osteogenesis activity[14]. Hydroxyproline is the unique composition of collagen, and detection of hydroxyproline content can reflect the synthetic collagen ability of BMSCs.

In this study, the expression level of ALP in the supernatant of pcDNA3.1-OGP transfected BMSCs was significantly higher than those in the vehicle and control groups. Hydroxyproline content was significantly higher in the pcDNA3.1-OGP transfected group. From the detection of these two osteoblast-specific markers, we considered that BMSCs were differentiated into osteoblasts under the direction of OGP expression. Thus we deducted that transfected BMSCs can express exogenous OPG gene stably.

Generally, this study constructed pcDNA3.1 plasmid successfully, transfected the plasmid into rabbit BMSCs and detected the transcription of OGP gene on the mRNA level. Transfected BMSCs expressed OGP mRNA and they have the characteristics of osteoblast-cells. This study may lay a

foundation for OGP gene therapy. In the future, we still need to work on the following issues: optimizing the purification of BMSCs, exploring the mechanism and conditions of signal transduction of osteoblast differentiation, improving the efficiency of target gene transfection and detecting the expression of OGP more directly. We believe that in the near future, with further studies in OGP, it can be used widely in the gene therapy of bone defects and nonunion.

REFERENCES

[1] Bruder SP, Jaiswal N, Ricalton NS, et al. Mesenchymal stem cells in osteobiology and applied bone regeneration. Clin Orthop Relat Res. 1998(355 Suppl):S247-256.
[2] Bab I, Gazit D, Chorev M, et al. Histone H4-related osteogenic growth peptide (OGP): a novel circulating stimulator of osteoblastic activity. EMBO J. 1992;11(5):1867-1873.
[3] Bab IA, Einhorn TA. Polypeptide factors regulating osteogenesis and bone marrow repair. J Cell Biochem. 1994; 55(3):358-365.
[4] Greenberg Z, Chorev M, Muhlrad A, et al. Mitogenic action of osteogenic growth peptide (OGP): role of amino and carboxy-terminal regions and charge. Biochim Biophys Acta. 1993;1178(3):273-280.
[5] Fei QM, Chen TY, Chen ZW, et al. Synthetic osteogenic growth peptide stimulates osteoblast osteogenic activity and enhances fracture healing in rabbits. Shengwu Huaxue yu Shengwu Wuli Xuebao. 2001;33(04):415-420.
[6] Chen XZ, Yang LJ, Yang WD. Combined applications of gene and tissue engineering in the restoration of bone defects. Xiandai Kangfu. 2001(5):59-61.
[7] Musgrave DS, Pruchnic R, Bosch P, et al. Human skeletal muscle cells in ex vivo gene therapy to deliver bone morphogenetic protein-2. J Bone Joint Surg Br. 2002;84(1): 120-127.
[8] Lin JR, Guo KG, Yang DG. A study on the clone culture of human bone marrow mesen chymecl stem cells and their exvivo support affect on hematopciesis. Zhongguo Xiandai Yixue Zazhi. 2003;13(6):8-11.
[9] Tsuda H, Wada T, Ito Y, et al. Efficient BMP2 gene transfer and bone formation of mesenchymal stem cells by a fiber-mutant adenoviral vector. Mol Ther. 2003;7(3):354-365.
[10] Tsuchida H, Hashimoto J, Crawford E, et al. Engineered allogeneic mesenchymal stem cells repair femoral segmental defect in rats. J Orthop Res. 2003;21(1):44-53.
[11] Bab I, Gazit D, Muhlrad A, et al. Regenerating bone marrow produces a potent growth-promoting activity to osteogenic cells. Endocrinology. 1988;123(1):345-352.
[12] Heim M, Frank O, Kampmann G, et al. The phytoestrogen genistein enhances osteogenesis and represses adipogenic differentiation of human primary bone marrow stromal cells. Endocrinology. 2004;145(2):848-859.
[13] Brager MA, Patterson MJ, Connolly JF, et al. Osteogenic growth peptide normally stimulated by blood loss and marrow ablation has local and systemic effects on fracture healing in rats. J Orthop Res. 2000;18(1):133-139.
[14] Sakano S, Murata Y, Miura T, et al. Collagen and alkaline phosphatase gene expression during bone morphogenetic protein (BMP)-induced cartilage and bone differentiation. Clin Orthop Relat Res. 1993(292):337-344.

pcDNA$_{3.1}$-成骨生长肽真核表达载体在骨髓基质干细胞中的表达***★

安　刚，吕松岑，郭亚山，薛　震，邓秋奎(哈尔滨医科大学附属第二医院骨科，黑龙江省哈尔滨市　150086)

安刚★，男，1982 年生，黑龙江省海林市人，汉族，2008 年哈尔滨医科大学毕业，硕士，医师，主要从事股骨头无菌性坏死的研究。
并列第一作者：郭亚山★，男，1972 年生，内蒙古自治区赤峰市人，汉族，2009 年哈尔滨医科大学毕业，硕士，主治医师，主要从事股骨头无菌性坏死的研究。
通讯作者：吕松岑，博士，教授，哈尔滨医科大学附属第二医院骨二科，黑龙江省哈尔滨市　150086

摘要

背景：成骨生长肽具有明显的促进成骨细胞增殖、分化、成熟的作用。

目的：观察成骨生长肽基因转染兔骨髓基质干细胞后的表达及表达产物对骨髓基质干细胞向成骨细胞分化的影响。

方法：构建重组真核表达载体 pcDNA$_{3.1}$-成骨生长肽，并在脂质体介导下，将其导入兔骨髓基质干细胞。通过 G418 筛选获得阳性克隆。RT-PCR 方法检测成骨生长肽基因在骨髓基质干细胞内的表达，并观察转染 pcDNA$_{3.1}$-成骨生长肽后骨髓基质干细胞向成骨细胞分化的情况。

结果与结论：实验成功构建了真核表达载体 pcDNA$_{3.1}$-成骨生长肽，转染 pcDNA$_{3.1}$-成骨生长肽的骨髓基质干细胞可见成骨生长肽 mRNA 的表达，同时羟脯氨酸的分泌水平增加，碱性磷酸酶活性增高。证实经 pcDNA$_{3.1}$-成骨生长肽转染的兔骨髓基质干细胞不仅可以表达成骨生长肽，而且具有向成骨细胞分化的特性。

关键词：成骨生长肽；pcDNA$_{3.1}$载体；骨髓基质干细胞；基因转染；表达
doi:10.3969/j.issn.1673-8225.2011.36.011
中图分类号: R394.2　文献标识码: A
文章编号: 1673-8225(2011)36-06696-05
安刚，吕松岑，郭亚山，薛震，邓秋奎. pcDNA$_{3.1}$-成骨生长肽真核表达载体在骨髓基质干细胞中的表达[J].中国组织工程研究与临床康复，2011，15(36):6696-6700.
[http://www.crter.org　http://cn.zglckf.com]
(Edited by Wang R/Qiu Y/Wang L)

来自本文课题的更多信息--

基金资助：黑龙江省留学回国人员基金 (LC04C03); 哈尔滨市青年科学研究基金、培养学科后备带头人基金、留学归国人员基金(2005AFLXJ016)。

作者贡献：安刚进行实验设计，实验实施为安刚、郭亚山，实验评估为吕松岑，资料收集为邓秋奎、薛震，安刚成文，吕松岑审校，吕松岑对文章负责。安刚与郭亚山对文章的贡献相同，故并列为第一作者。

致谢：感谢哈尔滨医科大学生化教研室给予的材料及技术上的支持。感谢哈尔滨医科大学附属第二医院实验中心和动物中心的工作人员在实验过程中给予的帮助。

利益冲突：课题未涉及任何厂家及相关雇主或其他经济组织直接或间接的经济或利益的赞助。

伦理批准：实验对动物的处理方法符合中华人民共和国科学技术部颁发的《关于善待实验动物的指导性意见》的相关要求。