Construction of adenovirus vectors carrying $VEGF_{121}$-FLAG and hrGFP-1 and their expressions in bone marrow stromal stem cells☆

Liu Dan-ping[1], Li Chen[1], Hu Liang[1], Wang Guo-xian[2]

Abstract

BACKGROUND: Vascular endothelial growth factor (VEGF) can promote angiogenesis, and has been extensively used in treatment of bone defect. However, few studies have addressed its isomer $VEGF_{121}$.
OBJECTIVE: To construct adenovirus vector carrying $VEGF_{121}$-FLAG and humanized Renilla reniformis green fluorescent protein 1(hrGFP-1) and observe its expression in bone marrow stromal stem cells (BMSCs).
METHODS: Using polymerase chain reaction technique, $VEGF_{121}$ gene contained in the plasmid of pTG19T-$VEGF_{121}$ was used to remove termination codon. *Not*I and *Xho* I restriction sites were added before and after gene sequence. Obtained gene subclone was moved onto pMD19-T plasmid. The pMD19-T-$VEGF_{121}$ and pShuttle-CMV-IRES-hrGFP-1 plasmids underwent double enzymatic digestion. Small fragment and big fragment were retrieved utilizing gel. Subsequently, coupled reaction was conducted to complete the construction of shuttle plasmid. After measuring virus titer, BMSCs were transfected and the fluorescence intensity was observed under fluorescence microscope.
RESULTS AND CONCLUSION: Recombinant adenovirus plasmid was successfully constructed by enzymatic digestion determination and gene sequence. Fluorescence microscope has shown that BMSCs transfected with recombinant adenovirus presented significantly green fluorescence expression. Thus, adenovirus vector carrying $VEGF_{121}$-FLAG and hrGFP-1 gene can express in eukaryotic cells, which can be used for gene therapy for ischemic disease.

[1]Department of Joint Surgery, First Affiliated Hospital, Liaoning Medical University, Jinzhou 121001, Liaoning Province, China; [2]Department of Pharmacology, Liaoning Medical University, Jinzhou 121001, Liaoning Province, China

Liu Dan-ping☆, Doctor, Professor, Department of Joint Surgery, First Affiliated Hospital, Liaoning Medical University, Jinzhou 121001, Liaoning Province, China liudanping2009@sohu.com

Correspondence to: Hu Liang, Master, Department of Joint Surgery, First Affiliated Hospital, Liaoning Medical University, Jinzhou 121001, Liaoning Province, China sasimi007@sina.com

Received: 2010-05-06
Accepted: 2010-06-17
(20100115016/D)

Liu DP, Li C, Hu L, Wang GX.Construction of adenovirus vectors carrying $VEGF_{121}$-FLAG and hrGFP-1 and their expressions in bone marrow stromal stem cells. Zhongguo Zuzhi Gongcheng Yanjiu yu Linchuang Kangfu. 2010;14(45):8539-8543.

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

INTRODUCTION

Factors that contribute to angiogenesis contain direct inducing factor and indirect inducing factor[1-3]. Vascular endothelial growth factor (VEGF) is a powerful vascular permeability factor. In particular, $VEGF_{165}$ and $VEGF_{121}$ effects are significant. $VEGF_{165}$ is half soluble and half insoluble proteins in a body, and can secrete *in vitro*. Some are combined with cell membrane or intercellular substance. However, $VEGF_{121}$ is completely soluble, does not bind with the weak acidic protein of heparin, completely secrete outside cells and floate in intercellular substance, can better exert paracrine effects, and promote angiogenesis[4-8]. Flt-1 receptor of VEGF could be found in osteoblasts; VEGF induced chemotaxis through strongly expressed Flt-1 receptor in osteoblasts. Thus, osteoblasts gathered in defect sites, and differentiated into osteogenic tissue affected by VEGF[9-25]. The present study used humanized Renilla reniformis green fluorescent protein (hrGFP-1) as report gene, constructed adenovirus expression system containing $VEGF_{121}$-FLAG and hrGFP-1 gene, and observed its expression in bone marrow stromal stem cells (BMSCs) *in vitro*, in the hope of providing experimental evidences for gene therapy for ischemic disease in the Department of Orthopaedics.

MATERIALS AND METHODS

Design

A cytological *in vitro* observational study.

Time and setting

Experiments were performed at the Central Laboratory First Affiliated Hospital, Liaoning Medical University from April 2008 to October 2009.

Materials

One clean male Japanese rabbit weighing 1.2 kg was supplied by the Laboratory Animal Center, Liaoning Medical University (animal license No. SCXK (Liao) 2003-2007). Protocols were conducted in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by Ministry of Science and Technology of the People's Republic of China[26].

Plasmid, strain, main reagents and kits are listed as follows:

Plasmid, strain, main reagent and kit	Source
pTG19T-$VEGF_{121}$ plasmid	Institute of East Hepatobiliary Surgery, Shanghai, China
pAdeasy-1, adenovirus shuttle plasmid pShuttle-CMV-IRES-hrGFP-1	Stratagene, USA
BJ5183 electroporated competence bacteria	Shanghai Genmed Gene Pharmaceutical Technology Co. Ltd., China
Pme Ⅰ and *Pac* Ⅰ enzyme various restriction enzyme, DNA Ligation Kit Ver.2.0, high-fidelity DNA polymerase, Agarose Gel DNA Purification Kit Ver.2.0 TaKaRa DNA Fragment Purification Kit Ver.2.0 plasmid small-volume extraction kit	New England Biolabs Inc., Beijing, China
dNTP, DNA marker	TaKaRa, USA

Methods

$VEGF_{121}$ gene mutation and pMD19-T plasmid subclone containing $VEGF_{121}$ gene following mutation (as +$VEGF_{121}$)

①Using polymerase chain reaction (PCR) technique, $VEGF_{121}$ gene contained in the plasmid of pTG19T-$VEGF_{121}$ was used to remove termination codon TAG sequence. *Not*I and *Xho* I restriction sites were added before and after gene sequence:

Primer sequence:

F: 5'-GCG GCC GCA TGA ACT TTC TGC TGT CTT G- 3'

R: 5' -CTC GAG CCG CCT CGG CTT GTC ACA TT- 3'

Using pTG19T-$VEGF_{121}$ as a template, PCR was performed; reaction condition is as follows: 98 ℃, 10 seconds for 30 cycles; 55 ℃, 5 seconds for 3 030 cycles; 72 ℃, 30 seconds for 30 cycles; 72 ℃, 5 minutes for 1 cycle. The samples were identified utilizing agarose gel electrophoresis.

② PCR products + "A" and purification: PCR products were retrieved. An "A" was added at the end of 3' of PCR products. The samples were than refined using TaKaRa DNA Fragment Purification Kit Ver.2.0.

③Connection, transformation, and screening of positive clone: Solution I of TaKaRa DNA Ligation Kit Ver.2.0 was employed to connect PCR products and pMD19-T plasmid. Competent cells were transfected, and flat plate was smeared, followed by incubation at 37 ℃ overnight.

④Plasmid extraction and sequencing.

Construction and identification of pShuttle CMV-$^{+}$$VEGF_{121}$-IRES-hrGFP-1

Plasmid pMD19-T-$^{+}$$VEGF_{121}$ and shuttle vector pShuttle-CMV-IRES-hrGFP-1 underwent *Not*I and *Xho*I double enzymatic digestion, and received agarose gel electrophoresis. Following gel retrieval, the samples were mixed with T4 DNA Ligase and buffer at molar ratio 10: 1 at 16 ℃ overnight. On the following day, the samples were transformed into bacteria DH5α, and clone was extracted. Following sequencing identification, target gene was inserted into shuttle vector using correct reading frame.

Adenoviral recombination and identification

Shuttle plasmid containing target gene was linearized using *Pme* I enzymatic digestion. pAdeasy-1 was transformed into BJ5183 bacteria to obtain BJ5183 bacteria containing pAdeasy-1. Correct pShuttle CMV- $^{+}$$VEGF_{121}$-IRES-hrGFP-1 was transformed into BJ5183 containing pAdeasy-1. Following mixture, the samples were added in a cup for electrotransformation. After electrotransformation, the medium supplemented with kanamycin was rapidly added in the pool, blew and hit evenly, overnight. A small colony was selected and amplified to extract plasmid. Following *Pac* Ⅰ enzymatic digestion, recombinant plasmid received agarose gel electrophoresis. A comparison with standard spectrum of recombinant adenovirus enzymatic digestion was done to identify the accuracy of obtained recombinant adenovirus.

Package and amplification of recombinant adenovirus

Package of adenovirus: 293A cells were incubated in a 6-well plate at 4×10^5. On day 2, recombinant adenovirus plasmid was linearized using *Pac* I enzymatic digestion, followed by gel retrieval and purification. Linearized plasmid DNA was transformed into 293T cells by using liposome lipofectamine 2000. On day 4, the medium was replaced. On day 11, cells were collected. Virus was collected following repeatedly freezing and thawing.

Amplification of adenovirus: 1/3 obtained virus was applied to infect new 293T cells. On 48 hours, cells were collected. A total of 4 amplifications were done. All cells were harvested, followed by repeatedly freezing and thawing, to obtain recombinant adenovirus.

Determination of physical titer of recombinant adenovirus

Recombinant adenovirus was greatly amplified, followed by twice $CsCl_2$ gradient centrifugation. After removal of $CsCl_2$ by dialysis, the samples were stored in buffer (10 mmol/LTris, 4% sucrose, 2 mmol/L $MgCl_2$, pH 8.0). Titer of Ad -$^{+}$$VEGF_{121}$-IRES-hrGFP-1 was measured by bacteriophage plaque analysis: 6.50×10^9 pfu/mL.

Collection and detection of bone marrow

Japanese rabbits were anesthetized with SU-MIAN-XIN II (0.2 mL/kg) by intramuscular injection. Internal side of inferior extremity of bilateral femurs was selected as puncture site. Following shaving hair, povidone iodine sterilization and paving aseptic towel, a total of 4.0 mL bone marrow was collected using No. 16 needle. On a superclean bench, 4.0 mL bone marrow was infused into 20 mL aseptic bottle, and incubated with 4.0 mL DMEM supplemented with 10% fetal bovine serum, and then triturated. 1.0 mL samples each were added in eight 30 cm^2 bottles, with an additional 3.0 mL DMEM, and finally incubated in an incubator containing 5% CO_2 at 37 ℃ for 5 days. The medium was replaced on day 5, and the medium was replaced every three days from then on. Cell growth was observed under an inverted microscope. The spindle-shape cells were BMSCs. Cells were subcultured 5 days later. Ad-$^{+}$$VEGF_{121}$ -IRES-hrGFP-1 was used to transfect the third passage of BMSCs, and gene expression was observed under a fluorescence microscope.

Main outcome measures

Enzymatic digestion pattern and sequencing site during the construction of adenovirus vector carrying $VEGF_{121}$-FLAG and hrGFP-1 genes were analyzed. Expression of recombinant adenovirus vector in BMSCs was measured.

Design, enforcement and evaluation

The first and third authors designed this study, collected data and performed this study. The first, second and fourth authors assessed results. They were professionally trained. The blind method was not used.

RESULTS

Package of recombinant adenovirus

Following Ad -$^{+}$$VEGF_{121}$-IRES-hrGFP-1 lipofectamine 2 000 cotransfection into 293T cells, cytopathic effects occurred at 2 weeks in most cells. 293 cells were repeatedly

infected, and 293T cells suffered from cytopathic effects at 3 days following the third infection, and fluorescence intensity became strong significantly (Figure 1).

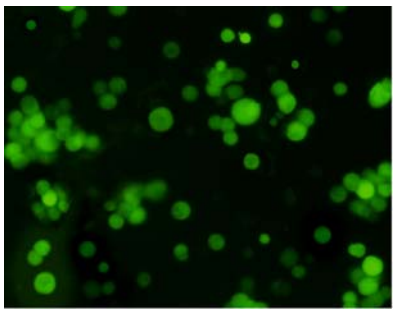


Figure 1 Strong fluorescence and cytopathic effects at 2 wk after transfection into 293T cells under fluorescence microscope

Gene expression in BMSCs

Strong green fluorescence could be detectable in the third passage of BMSCs at 48 hours following transfection of Ad-$^{+}$VEGF$_{121}$-IRES-hrGFP-1 under the fluorescence microscope (Figure 2).

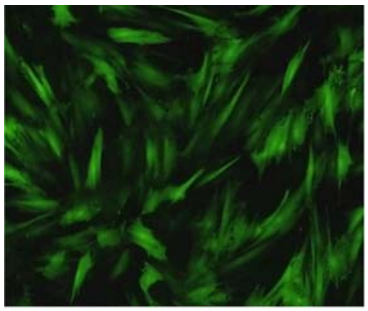


Figure 2 Strong fluorescence in bone marrow stromal stem cells at 48 h after transfection

Changes in pTG19T-VEGF$_{121}$ termination codon prior to and following mutation

Termination codon GTA was replaced by CTCGAG at *Xho*I locus following mutation (Figures 3, 4).

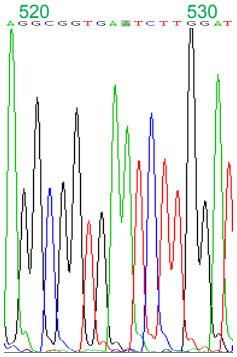


Figure 3 Partial sequencing map containing termination codon before mutation

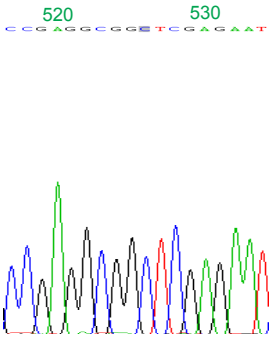
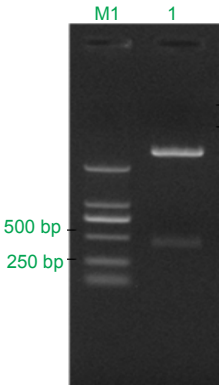


Figure 4 Partial sequencing map containing *Xho*I locus of termination codon removal after mutation

Electrophoresis results of pMD19-T-$^{+}$VEGF$_{121}$ following *Not*I and *Xho*I double enzymatic digestion

Target genes between 250 bp and 500 bp were visible (Figure 5).

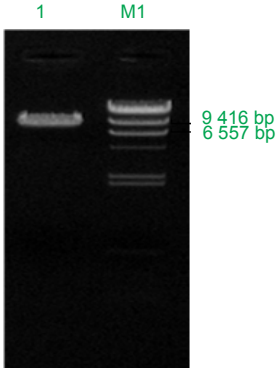


M1: DL2000 DNA Marker; 1: pMD19-T-$^{+}$VEGF$_{121}$ NotI/ XhoI

Figure 5 Electrophoresis result after pMD19-T-$^{+}$VEGF$_{121}$ was cleaved by *Not* Ⅰ and *Xho* Ⅰ

Electrophoresis results of pShuttle-CMV-IRES-hrGFP-1 following *Not*I and *Xho*I double enzymatic digestion

(Figure 6)



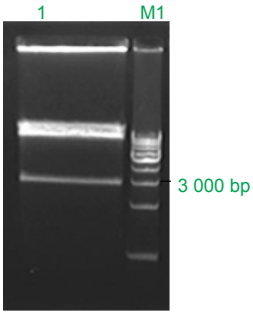
M1: λ-Hind Ⅲ digest DNA Marker; 1: pShuttle-CMV-IRES-hrGFP-1 *Not* Ⅰ/ *Xho* Ⅰ

Figure 6 Electrophoresis results after pShuttle-CMV-IRES-hrGFP-1 was cleaved by *Not* Ⅰ and *Xho* Ⅰ

Pac I enzymatic digestion results of pAd-$^{+}$VEGF$_{121}$-hrGFP-1

Two bands, 3 000 bp and 30 000 bp, comfirmed successful

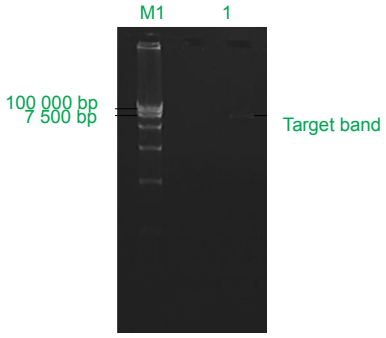
homologous recombination (Figure 7).



M1: 1 Kbp DNA Ladder Marker; 1: 3 000 bp and 30 000 bp following enzymatic digestion

Figure 7 Electrophoresis result of recombinant pAd-$^{+}$VEGF$_{121}$-hrGFP-1 cleaved by *Pac* Ⅰ

Electrophoresis results of pShuttle CMV-$^{+}$VEGF$_{121}$-IRES-hrGFP-1 (Figure 8)



M1: DL15000 DNA Marker; 1: pShuttle CMV-$^{+}$VEGF$_{121}$-IRES-hrGFP-1

Figure 8 Electrophoresis result of pShuttle CMV-$^{+}$VEGF$_{121}$-IRES-hrGFP-1

DISCUSSION

In the process of gene therapy, report gene-GFP was used to study intensity or time of exogenous gene expression *in vivo*. Enhanced GFP is commonly utilized to monitor *in vivo* gene expression and report gene of intracellular protein localization[27-28]. This study used hrGFP-1, which is twice lighter than enhanced GFP, can be easily detected, and has a small toxicity on mammalian cells. Following Ad-$^{+}$VEGF$_{121}$-IRES-hrGFP-1 transfection into BMSCs, target gene and hrGFP-1 transcription were on the same mRNA; internal ribosome IRES sequencing was bound with ribosome, which completed the simultaneous translation of target gene[29-32]. Utilizing PCR technique, VEGF$_{121}$ gene termination codon was removed. *Not*I and *Xho* I restriction sites were added before and after gene sequence. Gene can localize between *Not*I and *Xho*I of pShuttle CMV-IRES-hrGFP-1 multiple clone sites. FLAG antigen labeling sequence is visible prior to shuttle vector internal ribosome entry site sequencing. Following translation removal and termination codon, base sequence from VEGF$_{121}$ gene order translation initiation codon to FLAG epitope gene cannot lead to frameshift mutation of FLAG epitope gene, but result in read-through of two genes, and finally confluence of VEGF$_{121}$ and FLAG proteins. The isomers of VEGF contain six types, but lack of antibody of VEGF$_{121}$. Thus, FLAG antigen labeling is very important for VEGF$_{121}$ specificity test. As a vector of mediating VEGF gene, adenovirus is characterized by ① extensive host cells in adenovirus; infection on dividing and nondividing cells; ② high target gene expression; ③ carried gene cannot integrate into genome of host cells, without the risk of insertion mutation; ④ big adenoviral genome, whereas viral reproduction-needed cis element area is small[33-34]. Up to now, no reports have addressed that recombinant adenovirus lead to tumor or other genetic diseases. Thus, it can be utilized as a satisfactory vector for VEGF gene, and introduced into host cells to satisfy the requirement of VEGF during the treatment of ischemic disease in the Department of Orthopaedics. The present study successfully constructed recombinant adenovirus vector containing VEGF$_{121}$ and hrGFP-1 gene, and verified this recombinant adenovirus expressed target gene in eukaryotic cells. Therefore, author constructed recombinant adenovirus can be employed in following studies, and lay the foundation of *in vivo* local application of VEGF$_{121}$ gene therapy for ischemic disease.

REFERENCES

[1] Peng H, wright V, usas A, et al. Synergistic enhancement of bone formation and healing by stem cell expressed VEGF and bone morphogenetic protein 4. J Clin Invest. 2002;110(13):751-759.
[2] Zhang H, Tian XB, Sun L, et al. Expression of BMP2 and vegf165 genes simultaneity transfected mouse bone marrow mesenchymal stem cells. Guizhou Yiyao. 2009;33(5):392-395.
[3] Hu LL, Li XX, Zhang JY, et al. Expression products of chimeric bmp2 and bmp7 induce osteoblast differentiation. Tianjin Yiyao. 2009;37(10):820-822.
[4] He M, Cheng Y, Li W, et al. Vascular endothelial growth factor C promotes cervical cancer metastasis via up-regulation and activation of RhoA/ROCK-2/moesin cascade. BMC Cancer. 2010;10(1):170.
[5] Kinoshita Y, Kasaoka S, Fujita M, et al. Synchronized changes in serum vascular endothelial growth factor during the clinical course of chronic systemic capillary leak syndrome. Intern Med. 2010;49(8):791-794.
[6] Burger RA. Role of vascular endothelial growth factor inhibitors in the treatment of gynecologic malignancies. J Gynecol Oncol. 2010;21(1):3-11.
[7] Dvorak HF, Brown LF, Detmar M, et al. Vascular permeability factorvascular endothelial growth factor,microvascular hyperpermeability and angiogenesis. Am J Pathol. 1995; 146:1029-1039.
[8] Simons M, Bonow RO, Chronos NA, et al. Clinical trials in coronary anagiogenesis: issues problems consensus: An Expert Panel summary. Cireulation. 2000;102(11):E73- E86.
[9] Niida S, Kaku M, Amano H, et al.Vascular endothelial factor can substitute for macrophage colony-stimulating factor in the support of esteodastic bone resorption. J Exp Med. 1999; 190(2):293-298.
[10] Wang S, Miura M, Demura H, et al. Anabolic effects of 1,25-dihydroxy vitamin D3 on osteoblasts are enhanced by vascular endothelial growth factor produced by osteoblasts and by growth factors produced endothelial cells. Endocrinology. 1997;138:2953-2962.
[11] Mayr-wohlfart U, Waltenberger J, HousserH, et al. vascular endothelial growth factor stimulates chemotactic migration of primary human osteoblasts. Bone. 2002;30 (3):472-477.
[12] Gerber HP, Hillan KJ, Ryan AM, et al. VEGF is required for growth and survival in neonatal mice. Development. 1999; 126(6):1149-1159.
[13] Deckers MM, Karperien M, Bent C van der, et al. Expression of vascular endothelial growth factors and their receptors during osteoblast differentiation. Endocrinology. 2000;141(5): 1667-1674.

[14] Street J, Bao M, deGuzman L, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proc Natl Acad Sci USA. 2002;99(15):9656-9661.
[15] Gerber HP, Vu TH, Ryan AM, et al. VEGF couples hypertrophic cartilage remodeling,ossification andangiogenesis during endochondral bone formation. Nat Med. 1999;5(6):623-628.
[16] Goad DL, Rubin J, Wang H, et al.Enhanced expression of vascular endothelial growth factor in human SaOS-2 osteoblast-like cells and murine osteoblasts induced. by insulin-like growth factor I. Endocrinology. 1996;137(6):2262-2268.
[17] Harada S, Nagy JA, Sullivan KA, et al. Induction of vascular endothelial growth factor expression by prostaglandin E2 and E1 in osteoblasts. J Clin Invest. 1994;93(6):2490-2496.
[18] Steinbrech DS, Mehrara BJ, Saadeh PB, et al.VEGF expression in an osteoblast-like cell line is regulated by a hypoxia response mechanism. Am JPhysiol Cell Physiol. 2000;278(4):C853-860.
[19] Tsuboi M, Kaku M, Amano H, et al. Tumor necrosis factor-alpha and interleukin-1beta increase the Fas-mediated apoptosis of human osteoblasts. J Lab Clin Med. 1999;134(3):222-231.
[20] Hill PA, Tumber A, Meikle MC. Multiple extracellular signalspromote osteoblast survival and apoptosis. Endocrinology. 1997;138(9):3849-3858.
[21] Jilka RL, Weinstein RS, Bellido T, et al. Osteoblast programmed cell death (apoptosis): modulation by growth factors and cytokines. J Bone Miner Res. 1998;13(5):793-802.
[22] Forsythe JA, Jiang BH, Iyer NV, et al. Activation of vascular endothelial growth factor. gene transcription by hypoxia-inducible factor 1. Mol Cell Biol. 1996;16(9):4604-4613.
[23] Sun L, Tian XB, Yang SH, et al. In vivo ectopic osteogenesis of mouse bone marrow stromal stem cells transfected with bone morphogenetic protein 2 and vascular endothelial growth factor 165 genes. Zhonghua Zuzhi Gongcheng Yanjiu yu Linchuang Kangfu. 2008;12(47):9279-9282.
[24] Tian XB, Sun L, Zhang YK, et al. Construction and expression of a bicistronic vector containing human bone morphogenetic protein 2 and vascular endothelial growth factor-165 genes in vitro. Chinese Medical Journal. 2009;122 (4):471-473.
[25] Jiang J, Fan CY, Zeng BF. Co-transfection of rat bone marrow mesenchymal stem cells with human BMP2 ANDVEGF165. genes. 2009;21(1):12-18.
[26] 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.
[27] Chen J, Sai SY, Vazin T, et al. Human embryonic stem cells which express hrGFP in the undifferentiated state and during dopaminergic differentiation. Restor Neurol Neurosci. 2009;27(4):359-370.
[28] Salunkhe SS, Raiker VA, Rewanwar S, et al. Enhanced fluorescent properties of an OmpT site deleted mutant of Green Fluorescent Protein. Microb Cell Fact. 2010;9(1):26.
[29] Pelletier J, Sonenberg N. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature .1988;334(6180):320-325.
[30] Fitzgerald KD, Semler BL. Bridging IRES elements in mRNAs to the eukaryotic translation apparatus. Biochim Biophys Acta. 2009;1789(9-10):518-528.
[31] Filbin ME, Kieft JS. Toward a structural understanding of IRES RNA function. Curr Opin Struct Biol. 2009;19(3):267-276.
[32] Fernández-Miragall O, López de Quinto S, Martínez-Salas E. Relevance of RNA structure for the activity of picornavirus IRES elements. Virus Res. 2009;139(2):172-182.
[33] Lusky M, Christ M, Rittner K, et al. In vitro and in vivo biology of recombinant adenovirus vectors with E1, E1/E2A, or E1/E4 deleted. J Virol. 1998;72(3):2022-2032.
[34] Takeshita S, Zheng LP, Brogi E, et al. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. J Clin Invest. 1994;93(2):662-664.

构建携带 VEGF121-FLAG 和 hrGFP-1 基因腺病毒表达载体在骨髓基质干细胞中的表达☆

刘丹平[1]，李　谌[1]，胡　亮[1]，王国贤[2] ([1]辽宁医学院附属第一医院关节外科；[2]辽宁医学院药理学教研室，辽宁省锦州市　121001)

刘丹平☆，男，1961 年生，辽宁省锦州市人，汉族，解放军第四军医大学博士后，教授，主要从事骨组织工程相关领域研究。
通讯作者：胡亮，男，辽宁医学院硕士，主要从事骨组织工程相关领域研究。

摘要

背景：血管内皮生长因子具有促进血管生成的作用，在骨缺损的治疗研究中运用较多，但其异构体 VEGF121 的研究较少。

目的：构建携带 VEGF121-FLAG 和 hrGFP-1 基因的腺病毒表达载体并观测其在骨髓基质干细胞中的表达。

方法：以 pTG19T-VEGF121 为模板利用 PCR 技术突变 VEGF121 基因以去除 VEGF121 基因中终止密码子，之后在基因序列前后分别加入 *Not* Ⅰ和 *Xho* Ⅰ酶切位点，并将得到的基因亚克隆至 pMD19-T 质粒上，双酶切 pMD19-T-VEGF121 和 pShuttle-CMV-IRES-hrGFP-1 质粒，凝胶回收小片段和大片段，后进行连接反应，完成穿梭质粒的构建。重组病毒物理滴度测定后感染骨髓基质干细胞，在荧光显微镜下观察荧光强度。

结果与结论：经酶切鉴定及基因测序证实重组腺病毒质粒构建成功，荧光显微镜下观察表明，感染重组腺病毒的骨髓基质干细胞有明显的绿色荧光表达。可见构建的携带 VEGF121-FLAG 和 hrGFP-1 基因的腺病毒表达载体可在真核细胞表达，有可能用于缺血性疾患的基因治疗。

关键词：骨缺损；VEGF121；hrGFP-1；腺病毒载体；绿色荧光蛋白；基因治疗
doi:10.3969/j.issn.1673-8225.2010.45.042
中图分类号: R394.2　文献标识码: B
文章编号: 1673-8225(2010)45-08539-05
刘丹平，李谌，胡亮，王国贤. 构建携带 VEGF121-FLAG 和 hrGFP-1 基因腺病毒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，2010，14(45):8539-8543.
[http://www.crter.org　http://cn.zglckf.com]
(Edited by Zhang N/Qiu Y/Wang L)