



Construction of an adenoviral expression vector carrying FLAG and hrGFP-1 genes and its expression in bone marrow mesenchymal stem cells

G.X. Wang¹, L. Hu², Z. Zhang³ and D.P. Liu³

¹Department of Pharmacology, Liaoning Medical University, Jinzhou, Liaoning, China

²Department of Orthopaedics, First People's Hospital of Jingmen, China

³Department of Bone and Joint Surgery, First Affiliated Hospital of Liaoning Medical University, Jinzhou, China

Corresponding author: D.P. Liu

E-mail: liudpcn@126.com

Genet. Mol. Res. 13 (1): 1070-1078 (2014)

Received January 18, 2013

Accepted July 12, 2013

Published February 20, 2014

DOI <http://dx.doi.org/10.4238/2014.February.20.8>

ABSTRACT. The aim of this study was to construct an adenoviral expression vector for vascular endothelium growth factor 121 (VEGF₁₂₁)-FLAG and humanized *Renilla reniformis* green fluorescent protein (hrGFP-1) genes, and to observe their expressions in bone marrow mesenchymal stem cells. Using pTG19T-VEGF₁₂₁ as a template, polymerase chain reaction technology was adopted to mutate the VEGF₁₂₁ gene by removing the stop codon and inserting *NotI* and *XhoI* restriction sites both before and after the gene sequences. The resultant gene was then subcloned into a pMD19-T plasmid, the pMD19-T-VEGF₁₂₁ and pShuttle-CMV-IRES-hrGFP-1 plasmids were double-digested, and small and large fragments were linked after gel recovery to complete the construction of recombinant adenovirus vectors. After titer determination, the recombinant adenovirus vectors were used to affect

rabbit bone marrow mesenchymal stem cells, and fluorescence intensity was observed under fluorescence microscopy. Enzyme digestion identification and sequencing confirmed that the recombinant plasmids were successfully constructed, and observations under fluorescence microscopy showed significant expression of green fluorescent protein in recombinant adenovirus-infected bone marrow mesenchymal stem cells. The constructed adenoviral gene expression vectors carrying VEGF₁₂₁-FLAG and hrGFP-1 can be expressed in eukaryotic cells, which may be used for gene therapy of ischemic disorders.

Key words: Bone defect; Vascular endothelial growth factor 121; Adenovirus vector; Green fluorescent protein