INTRODUCTION

Vascular endothelial growth factor (VEGF) is one of the key regulators of angiogenesis. Angiogenesis is a crucial event during endochondral ossification. Deficiencies in vascularity and osteogenesis will lead to a delayed union, nonunion or necrosis. VEGF may play key roles in the regulation of angiogenesis at sites processing endochondral bone formation. The local release of VEGF from the osteogenic cells at the site near the necrotic bone may also enhance the repair of osteonecrosis. Previous reports indicated that VEGF-induced angiogenesis resulted in a therapeutic benefit in several animal models of limb ischemia. We propose that the transplantation of osteoprogenitor cells transfected with VEGF gene would be beneficial for the therapy of fracture repair and osteonecrosis. This study was designed to transfect the VEGF gene into the pluripotent stem cell (D1-cell), and test the potential for gene therapy in vitro. We examined the expressions of mRNA and protein of VEGF, and its bioactivity in D1 cells that transfected with VEGF containing vector.

METHODS

Cell culture: D1-cells, which have been cloned from bone marrow of Balb/c mouse, own an important property of being capable of homing to bone and accumulating at the bone repair site. D1-cells were maintained in Dulbecco Modified Eagle Medium containing 10% fetal bovine serum, and 50 mg/ml sodium ascorbate.

Construction of pIREShyg2-VEGF: Full length Mouse VEGF-cDNA was cloned from D1 cells by RT-PCR. The cDNA was amplified by PCR. A thermostable DNA polymerase, AmpliTag Gold (Perkin-Elmer), was used for the reaction. PCR product was analyzed by electrophoresis on an agarose gel and purified for cloning. The pIREShyg2 is a CMV promoter driven vector encodes a hygromycin resistance protein (Hyg^r), allowing long-term expression of the transgene in mammalian cells.

Transfection of D1 cells with pIREShyg2-VEGF: Stable transfection was performed by using electroporation. Transfectants was then be selected by hygromycin.

Assay of stable transfected osteoprogenitor cells: The retention of osteoblastic property of the transfected cells was analyzed by measuring expression of osteocalcin mRNA. The effective expression of biologically active VEGF was examined by RT-PCR for mRNA expression, Western blot analysis for the protein levels, and the proliferation of endothelial cells for bioactivity.

RESULTS

The efficiency of transfection method was evaluated by transfected the pIREs2-EGFP vector containing VEGF sequence into D1-cells. Electroporational delivery for transfection revealed more efficient than that with lipofectin reagent. The D1-cells transfected with pIREShyg2-VEGF have been selected with hygromycin. Our result showed that the VEGF transfected D1-cells preserve the osteogenic potential to express similar level of osteocalcin as vector control and non-transfected D1-cells(Fig.1). VEGF transfected D1-cells expressed significant higher level of mRNA and protein of VEGF than control cells (Fig.2, 3). The supernatant of VEGF transfected D1-cells significantly stimulated the proliferation of endothelial cells, revealing the released VEGF possessed the bioactivity of angiogenesis (Fig. 4).

DISCUSSION

In this study, we have successfully constructed a vector of pIREShyg2-VEGF, transfected into D1-cells by electroporation, and selected with hygromycin for long-term expression of VEGF. Our results showed that the transfected cells expressed the significant higher level of mRNA, protein and bioactivity of VEGF in comparison with the vector control or non-transfected D1-cells. These results showed that the VEGF transfected D1-cells could stably produce the bioactive VEGF. Our results also revealed that the transfected D1-cell still preserve the osteogenic characteristic. This phenomenon ensures that the transfection does not change the phenotype or the capacity of the cell to differentiation into osteoblasts. These results suggested that VEGF transfected D1-cell can be used as the carrier for gene therapy of fracture repair and osteonecrosis. In this study, the stable-transfected cells were tested to evaluate the potential of angiogenesis and osteogenesis for the further study of gene therapy. Transient-transfected cells may be used in future studies.

REFERENCES