INTRODUCTION

Distraction osteogenesis (DO) has become a widely used and accepted procedure for limb lengthening in patients with bone defects, limb length discrepancies, limb deformities, and short stature. Although DO is a well-established technique for bone lengthening, one of the problems is the long external fixation period, which can cause significant morbidity to the patient. Good bone formation during DO is essential to minimize the treatment time and reduce associated complications. We have demonstrated that transplantation of culture expanded bone marrow derived mesenchymal stem cells (BMSCs) into the distracted callus promoted new bone formation and shortened the consolidation period clinically and experimentally. 1-2

Angiogenesis, in which vascular endothelial growth factor (VEGF) has an important role, is an essential component of bone healing and regeneration. VEGF can interact synergistically with BMSCs to promote bone formation and bone healing by increasing angiogenesis. In this study, we have established the VEGF gene transfected BMSCs and transplanted these cells into the distracted gap of the rat DO model to evaluate the efficacy of the VEGF for bone regeneration.

MATERIALS AND METHODS

BMSCs were obtained from the femora of 8-week old Sprague-Dawley (SD) rats. Adherent cells were expanded as monolayer cultures in a 5% CO₂/95% air atmosphere at 37 °C with medium changes every 3 days. After 1 week in primary culture, cells were suspended using trypsin-EDTA, counted with a blood counter, and subcultured in the polystyrene multiwell plates (1x10^5 cell per well). Four days after subculture, full-length VEGF cDNA (pCAhVEGF) was transfected into BMSCs with LIPOFECT AMINE® 2000 Reagent. Transfection efficiency in BMSCs was evaluated by measuring the VEGF protein within the culture medium.

Nine-week-old male Sprague-Dawley rats weighing 320 to 380 g were used for DO experiments. After 7 days, the lengthening of the femur was initiated at a rate of 0.35 mm per 12 hours for 10 days. Culture expanded BMSCs with collagen gel were transplanted into the distracted callus during distraction period or immediately after the termination of distraction. Two groups were prepared – VEGF (+) : VEGF gene transfected BMSCs, and VEGF(-)BMSCs without VEGF.

Radiographs of the distracted femurs were taken at just after transplantation, 1, 2, 3 and 4 weeks after transplantation with a soft X-ray apparatus. The rats were sacrificed at just after transplantation, 1, 2, 3 and 4 weeks after transplantation. The specimens were fixed in paraformaldehyde, and embedded in paraffin. For immunohistochemistry, serial sections were used for detection of VEGF with specific antibody.

RESULTS

VEGF concentration within the culture medium of the VEGF(+) cells showed basal level, while increased VEGF concentration was observed in the VEGF(+) cells (Fig. 1).

Radiological examination of the distracted callus was shown in Figure 2. Callus formation was enhanced by the transplantation of VEGF transfected BMSCs during consolidation period (Fig. 2 A, B). The effect of promoting new bone regeneration was prominent when the cells were transplanted during distraction period (Fig. 2 C, D).

Immunohistochemical examinations demonstrated that VEGF expression within the new bone regenerates was increased in the VEGF(+) group (Fig. 3).

DISCUSSION

It is suggested that neovascularization of the distraction gap is essential for successful bone lengthening, and VEGF may also play crucial roles in DO in addition to bone growth factors. In this study, new bone formation was enhanced radiologically and elevated expression of the VEGF was confirmed immunohistologically in the VEGF(+) group compared to the VEGF(-) group. Application of VEGF may be considered as a potential method to enhance angiogenesis and osteogenesis in DO.

Previous studies demonstrated that expression of several growth factors that promote osteogenesis, such as TGF-beta, IGF-1, and basic FGF, was higher in the distraction phase than in the consolidation phase.

In this study, new bone formation was accelerated when the VEGF transfected BMSCs were transplanted during the distraction phase. The expression of endogenous growth factors and their receptors that accelerate osteogenesis may be elevated in this active phase.

REFERENCES