



Recombinant adeno-associated virus BMP-4/7 fusion gene confers ossification activity in rabbit bone marrow stromal cells

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ABSTRACT. The biological effects of transfection of an adeno-associated virus (AAV) vector with bone morphogenetic proteins 4 and 7 (BMP-4/7) fusion gene (AAV-BMP-4/7) were determined in rabbit bone marrow stromal cells (BMSCs). BMP-4 and BMP-7 genes were obtained through one-step reverse transcriptase polymerase chain reaction from human placental cells. The BMP-4/7 fusion gene was then generated through recombination. Rabbit BMSCs were transfected with the recombinant AAV vectors carrying AAV-BMP-4/7 with multiplicity of infection values. Cell growth curves were drawn to evaluate the biological effects of AAV-BMP-4/7 on cell activity. The transfection efficiency was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The ossification of cells was evaluated by observing alkaline phosphatase (ALP) and osteocalcin (OC) activity after transfection for 7 and 14 days. The cells were then transfected with AAV-BMP-4/7 and AAV-enhanced green fluorescent protein. We successfully constructed the recombinant adeno-associated virus with the BMP-4/7 fusion gene. The transfection efficiency of AAV-BMP-4/7 was approximately 72% without significant biological effects on cell activity. Cell ossification was significant after

transfection with AAV-BMP-4/7. The 1×10^5 vg/cell multiplicity of infection value of transfection efficiency was more than 5×10^4 vg/cell (59.38%). Significantly higher ALP and OC activity occurred in the AAV-BMP-4/7 transfection groups than in the AAV-enhanced green fluorescent protein groups ($t_{ALP} = 896.88$, $P < 0.001$; $t_{OC} = 543.24$, $P < 0.01$). The AAV-BMP-4/7 fusion gene can highly efficient transfect rabbit BMSCs cultured *in vitro* and it has significant ossification activity.

Key words: Bone tissue engineering; Bone morphogenetic protein; Fusion gene; Adeno-associated virus; Bone marrow stromal cells