

论著

改良腺病毒AdF35-eGFP转染人及大鼠骨髓间充质干细胞的效率对比研究

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摘要:

目的: 比较改良腺病毒AdF35-增强绿荧光蛋白(eGFP)转染人骨髓间充质干细胞(human bone marrow mesenchymal stem cells, hBMSCs)和 大鼠骨髓间充质干细胞(rat BMSCs, rBMSCs)的效率。方法: 分别从人体、大鼠骨髓中分离出BMSCs, 成骨、成脂诱导培养以鉴定BMSCs。构建含eGFP的AdF35重组腺病毒载体AdF35-eGFP, 以不同感染复数(multiplicity of infection, MOI)分别转染BMSCs后, MTT检测AdF35-eGFP对2种BMSCs的毒性作用, 荧光显微镜观察eGFP的表达, 流式细胞仪检测转染效率, 实时定量PCR检测2种BMSCs表达柯萨奇腺病毒受体(CAR)和CD46 mRNA的水平。结果: hBMSCs和rBMSCs从骨髓中分离出来后, 分别成功诱导分化成骨和成脂。当MOI为1 000 PFU/mL时, AdF35-EGFP对2种BMSCs的活性均有明显抑制作用(P<0.001)。AdF35-eGFP感染hBMSCs 48 h后, 荧光显微镜下可见发强烈绿色荧光的细胞, 流式细胞仪检测其转染效率可达(84.8±7.1)%; Ad5-eGFP感染rBMSCs后, 荧光显微镜下仅见少量发绿色荧光的细胞, 48 h转染效率为(3.1±1.1)%。hBMSCs高表达CD46 mRNA, 低表达CAR mRNA; 而rBMSCs则高表达CAR mRNA, 低表达CD46 mRNA, 2种基因的表达差异有统计学意义(P<0.01)。结论: AdF35可作为理想载体携带目的基因转染hBMSCs, 但不适合作为转染rBMSCs的载体。

关键词: 35型腺病毒 骨髓间充质干细胞 转染 CD46 柯萨奇腺病毒受体

Efficiency of human and rat bone marrow mesenchymal stem cells transfected by modified adenovirus

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Abstract:

Objective To compare the efficiency of human bone marrow mesenchymal stem cells (hBMSCs) with rat BMSCs (rBMSCs) transfected by modified adenovirus containing fiber 35 (AdF35)-enhanced green fluorescence protein (eGFP). Methods We separated hBMSCs and rBMSCs from the bone marrow of humans and rats, respectively, and osteogenesis and adipogenesis were induced. eGFP was carried by modified AdF35, which was transfected to hBMSCs and rBMSCs with different multiplicity of infections (MOIs). Activity of the cells was detected by MTT. The transfected cells were observed under fluorescent microscope. The transfection efficiency was measured by flow cytometer. The expression of coxsackie and adenovirus receptor (CAR) and CD46 mRNA in the cells was inspected by real time PCR. Results hBMSCs and rBMSCs induced osteogenesis and adipogenesis successfully after being separated from human and rat bone marrow respectively. The activity of the cells was inhibited when MOI was 1 000 PFU/mL. hBMSCs with strong green fluorescence were observed but few rBMSCs were seen under fluorescence microscope 48 h after being transfected by AdF35-eGFP. The transfective efficiency was (84.8±7.1)% and (3.3±1.1)%, respectively. The expression of CD46 was high while that of CAR was low in hBMSCs. The expression of CAR was very high and that of CD46 was low in rBMSCs (P<0.01). Conclusion AdF35 may be the ideal vector to carry the target gene to transfect hBMSCs effectively but not to transfect rBMSCs.

Keywords: adenovirus containing fiber 35; bone marrow mesenchymal stem cell; transfection; CD46; coxsackie and adenovirus receptor

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