

论著

重组腺病毒转染大鼠骨髓间质干细胞的研究

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摘要 目的: 观察腺病毒载体转染骨髓间质干细胞(BMSCs)的有效性和外源性基因表达的时相性。方法: 体外培养大鼠骨髓间质干细胞, 用已构建好的Ad5-CMV- GFP真核载体分别转染培养的第3代(P3)及第8代(P8) BMSCs。流式细胞仪检测转染后第2、4、7及10 d绿色荧光蛋白(GFP)的表达率。RT-PCR、Western杂交等方法检测腺病毒受体(CAR)在各代BMSCs中的基因和蛋白质的表达。结果: 重组腺病毒对BMSCs的转染率可达80%, P3与P8代BMSCs转染率无明显差异($P>0.05$)。转染后2 d可见GFP表达, 第7 d表达达高峰, 28 d仍可见GFP在BMSCs中表达。CAR在P1明显低于P2、P3、P6、P8各代BMSCs($P<0.05$), 但P3、P6与P8 BMSCs表达的CAR无显著差别($P>0.05$)。结论: 重组腺病毒载体能有效转染骨髓间质干细胞, 并维持1个月左右。P3与P8 BMSCs均可作为基因治疗的高效分子载体。

关键词 [腺病毒](#) [基因转移](#) [基因表达](#) [骨髓](#); [间质干细胞](#)

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Gene transfer into different passages of bone marrow mesenchymal stem cells

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Abstract

AIM: To investigate the efficiency and stability of adenovirus-mediated gene transfer into different passages of bone marrow mesenchymal stem cells (BMSCs). METHODS: BMSCs were obtained from bone marrow of SD rats and cultured. Then passage 3 (P3) and P8 BMSCs were transfected with Ad-CMV-GFP, respectively. The transfection ratio was evaluated by flow cytometry. At the same time coxsackie and Ad receptor (CAR) of different passages of BMSCs was estimated by RT-PCR and Western blotting. RESULTS: The green fluorescence was observed 24 h after transfection, while the strength of fluorescence increased with time and the peak was at 7 days. It was seen that the transfection ratio was over 80% and there was no difference between P3 and P8 BMSCs ($P>0.05$). Flow cytometry analysis by different gates showed the transfection ratio was high in BMSCs in the period of productive metabolism. The mRNA expression of CAR in P3, P6 and P8 was similar, and the same change was observed in the protein expression of CAR in P3 and P8 BMSCs. CONCLUSION: Ad-CMV-GFP is transferred to BMSC effectively and sustained about 28 days. It is suspected that BMSCs in mitotic phase are easy to be transferred by Ad-CMV-GFP and different passages of BMSCs from P3 to P8 BMSCs can be as high-effectively gene vehicle.

Key words [Adenoviruses](#) [Gene transfer](#) [Gene expression](#) [Bone marrow](#) [Mesenchymal stem cells](#)

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