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Title: Effect of SDF-1/HOXB4 fusion gene-modified mesenchymal stem cells on amplification and stemness maintenance of hematopoietic stem cells *in vitro*

作者: [张佩](#); [陈婷婷](#); [叶枫](#); [裴莉](#); [符刚](#); [徐双年](#); [邹仲敏](#); [张勇](#)
第三军医大学: 西南医院血液科, 军事预防医学院毒理学研究所

Author(s): [Zhang Pei](#); [Chen Tingting](#); [Ye Feng](#); [Pei Li](#); [Fu Gang](#); [Xu Shuangnian](#); [Zou Zhongmin](#); [Zhang Yong](#)

Department of Hematology, Southwest Hospital, Institute of Toxicology, Third Military Medical University, Chongqing, 400038, China

关键词: [腺病毒载体](#); [间充质干细胞](#); [造血干细胞](#); [共培养](#); [融合蛋白](#)

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摘要: 目的 构建SDF-1、HOXB4和 SDF-1/HOXB4融合基因腺病毒表达载体, 转染体外培养的间充质干细胞(mesenchymal stem cells, MSCs), 比较其对CD34⁺细胞体外扩增及干性维持的影响。 方法 全基因合成SDF-1/HOXB4基因序列, 以其为模板, 经PCR、酶切及测序鉴定, 构建表达SDF-1、HOXB4和SDF-1/HOXB4的3种腺病毒载体, 转染至293A细胞进行包装和病毒滴度测定, 并转染体外培养的MSCs, 分别为SDF-1组、HOXB4组和S-H组, 转染空腺病毒载体的为阴性对照, 转录水平及蛋白水平检测MSCs中外源基因表达。4组MSCs和CD34⁺细胞共培养7 d, 计数细胞并检测其CD34⁺表达。 结果 测序表明3种腺病毒载体构建成功, 在293A细胞中成功包装并获取病毒, RT-PCR和Western blot检测3个基因在MSCs细胞中稳定高表达, MSCs和CD34⁺细胞共培养, 扩增细胞数目SDF-1组(9.52±2.24)、S-H组(8.11±2.34)显著高于对照组(4.85±2.53, P<0.05), S-H组(CD34⁺表达为1.85%)较SDF-1组(1.20%)、HOXB4组(1.28%)更利于CD34⁺细胞干性维持。 结论 表达SDF-1/HOXB4融合基因的MSCs对CD34⁺细胞体外扩增及干性维持作用更好。

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Abstract: Objective To establish mesenchymal stem cells (MSCs) expressing SDF-1, HOXB4 and SDF-1/HOXB4 fusion gene respectively, and to compare their effects on the amplification and stemness maintenance of umbilical cord blood CD34⁺ cells *in vitro*. Methods The SDF-1/HOXB4 fusion gene was synthesized, based on which SDF-1 and HOXB4 gene were obtained by PCR, and adenovirus vectors respectively expressing above gene fragments were constructed. Virus particles were packaged in 293A cells and the titer of the virus was determined. MSCs infected with different virus supernatants were divided into a SDF-1 group, a HOXB4 group and a SDF-1/HOXB4 fusion gene group (S-H group), and MSCs infected with the adenovirus lack of target genes was used as control. The mRNA and protein expression of exogenous genes were detected. The 4 groups of MSCs were co-cultured with umbilical cord blood CD34⁺ cells for 7 d, and then total cell counting and the ratio of CD34⁺ cells were measured. Results The adenovirus vectors respectively expressing SDF-1, HOXB4 and SDF-1/HOXB4 were successfully constructed, and the MSCs infected with harvested virus supernatants stably and highly expressed the exogenous genes. The modified MSCs of the SDF-1 group (9.52 ± 2.24) and S-H group (8.20 ± 2.34) could significantly increase the total number of hematopoietic cells as compared with the control group (4.85 ± 2.53 , $P < 0.05$), and the S-H group (the proportion of CD34⁺ cells 1.85%) showed better effect on stemness maintenance of umbilical cord blood CD34⁺ cells as compared with the SDF-1 group (1.20%) and HOXB4 group (1.28%). Conclusion The SDF-1/HOXB4 fusion gene-modified MSCs can improve the amplification and stemness maintenance of HSCs *in vitro*.

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