中国医学科学院学报 2008 30 (4): 498-505 ISSN: 1000-503x CN: 11-2237/R

论著

人单纯疱疹病毒1型VP22介导的microdystrophin重组腺病毒的构建及其蛋白转导特性

熊 符[1,2], 张 成[2], 郑 卉[2], 肖少波[3], 潘永飞[3], 许勇峰[2], 刘正山[2], 李 勇[2]

[1]南方医科大学医学遗传学教研室，广州 510515;

[2]中山大学 附属第一医院神经内科，广州 510080;

[3]华中农业大学农业微生物国家重点实验室，武汉 430070

收稿日期 2007-9-11 修回日期 网络版发布日期 2008-9-3 接受日期

摘要 摘要：目的 构建含有人单纯疱疹病毒1型（HSV-1）VP22与人microdystrophin基因融合的重组腺病毒，体外感染成肌细胞C2C12，探讨VP22对microdystrophin基因在成肌细胞中的蛋白转导特性。方法 设计引物，从质粒pSINrep5-VP22中扩增VP22全长基因，然后将VP22基因定向插入腺病毒穿梭质粒pShuttle-CMV，获得重组质粒pCMV-VP22;再用Not Ⅰ酶切含microdystrophin基因的pBSK-micro质粒，获得microdystrophin基因，片段回收后定向插入重组质粒pCMV-VP22，获得重组质粒pCMV-VP22-MICDYS。PmeI线性化重组质粒pCMV-VP22-MICDYS，去磷酸化后回收与腺病毒骨架质粒pAdeasy-1共电转化BJ5183感受态细胞。同源重组后用选择性培养基筛选阳性克隆，提取质粒，脂质体介导转染293细胞，通过观察293细胞病变及PCR扩增目的基因等方法鉴定重组腺病毒。将含VP22-microdystrophin及microdystrophin的病毒上清分别转染成肌细胞C2C12，通过RT-PCR、Western-blot和免疫组织化学方法检测microdystrophin的mRNA及蛋白表达。结果 成功构建了含有VP22-microdystrophin基因的重组腺病毒,体外感染成肌细胞C2C12，Western-blot及免疫组织化学检测显示VP22显著提高了microdystrophin蛋白在C2C12细胞中的表达。结论 含有VP22-microdystrophin基因的重组腺病毒载体的构建及VP22介导microdystrophin在成肌细胞C2C12间的转导，为利用此病毒进行假肥大型肌营养不良症疾病的治疗研究奠定了基础。

关键词 VP22 microdystrophin 腺病毒 假肥大型肌营养不良症

分类号

Construction of Herpes Simplex Virus-1 Virion Protein 22-mediated Microdystrophin Gene Recombinant Adenovirus and Study on the Property of Protein Transduction

XIONG Fu[1，2],ZHANG Cheng[2],ZHENG Hui[2],XIAO Shao-bo[3],PAN Yong-fei[3],XU Yong-feng[2],LIU Zheng-shan[2],LI Yong[2]

[1]Department of Medical Genetics，Southern Medical University, Guangzhou 510515,China;

[2]Department of Neurology , the First Affiliated Hospital , Sun Yat-sen University，Guangzhou 510080,China;

[3]Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070，China

Abstract ABSTRACT:Objective To construct the recombinant adenovirus containing herpes simplex virus-1 virion protein (VP) 22 and human microdystrophin gene, then the adenovirus was transfected into C2C12 myoblast and studied on the property of protein transduction with VP22-mediated microdystrophin in C2C12 myoblast. Methods The full-length VP22 cDNA was obtained from recombinant plasmid pSINrep5-VP22 with PCR, and the product was directionally inserted into pShuttle-CMV to acquire the plasmid pCMV-VP22. Microdystrophin cDNA was obtained from recombinant plasmid pBSK-micro digested with restrictive endonuclease NotI, and the product was directionally inserted into pCMV-VP22 to acquire the plasmid pCMV-VP22-MICDYS. The plasmid of pCMV-VP22-MICDYS was lined with Pme Ⅰ, and

the fragment containing VP22-microdystrophin was reclaimed and transfected into E1 coli BJ5183 with plasmid pAdeasy-1. After having been screened by selected media, the extracted plasmid of positive bacteria was transfected into HEK293 cells with liposome and was identified by observing the cytopathic effect of cells and by PCR method to acquire the recombinant adenovirus Ad-VP22-MICDYS. Finally, the C2C12 myoblast were transfected with the recombinant adenovirus Ad-VP22-MICDYS and Ad-MICDYS, and the expression of microdystrophin was detected by RT-PCR, Western blot and immunocytochemistry. Results The recombinant adenovirus including VP22 and microdystrophin gene was successfully constructed. VP22 transferred VP22-microdystrophin fused protein from infected C2C12 myoblast into uninfected cells and enhance the expression of microdystrophin in myoblast. Conclusions Recombinant adenovirus containing VP22 and microdystrophin gene was constructed successfully. VP22 can enhance the expression with microdystrophin in myoblast. It lays the foundation for further studying on VP22-mediated recombinant including microdystrophin gene to cure Duchenne muscular dystrophy.

Key words virion protein 22 microdystrophin adenovirus Duchenne muscular dystrophy

DOI:

通讯作者 张 成 zhangch6@mail.sysu.edu.cn