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论著

腺病毒介导反义RNA抑制HIV-1辅助受体CCR5和CXCR4表达

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摘要 摘要：目的 抑制细胞表面人免疫缺陷病毒1型（HIV-1）的辅助受体CCR5和CXCR4表达,阻止HIV-1进入靶细胞。方法 逆转录聚合酶链反应扩增HIV-1辅助受体基因5〖WT《TNR》〗’-端cDNA片段,反向插入穿梭质粒中,再与腺病毒基因组骨架质粒在大肠杆菌 BJ5183中同源重组,重组质粒在293细胞中包装、扩增得到含有〖STBX〗CCR5、CXCR4〖ST〗反义RNA的重组腺病毒,分别感染U937细胞和MT4细胞,于感染后24、48和72 h及10 d时用流式细胞仪检测细胞表面相应受体的表达。并用Boyden小室法检测重组腺病毒感染对细胞趋化活性的影响以及用3H掺入法检测对细胞增殖能力的影响。结果 成功获得含有〖STBX〗CCR5和CXCR4〖ST〗反义RNA的重组腺病毒,其滴度为5×1011 PFU/ml和7×1010 PFU/ml。重组腺病毒感染24 h后流式细胞仪检测显示,U937细胞表面CCR5的表达率分别为：Ad-antiR5 1.12%,未感染细胞对照82.10%,Ad-senseR5 80.94%,Ad-control 81%。MT4细胞表面CXCR4的表达率分别为：Ad-antiX4 1.03%,未感染细胞对照42%,Ad-senseX4 44%,Ad-control 39%。48、72 h及10 d后Ad-antiR5感染的U937细胞表面CCR5表达率分别为：1.02%、1.26%、1.23%;Ad-antiX4感染的MT4细胞表面CXCR4表达率分别为：1.13%、1.17%、1.22%。感染重组腺病毒后,两种细胞的趋化活性及增殖能力均无改变。结论 包含有CCR5、CXCR4反义RNA的重组腺病毒可以使细胞表面相应受体表达下降,且重组腺病毒不影响细胞的趋化活性和增殖能力。

关键词 重组腺病毒 辅助受体 反义RNA 抑制

分类号

Inhibition of Expression of CCR5 and CXCR4 on Cells by Adenovirus-mediated Antisense RNA

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Abstract

ABSTRACT:Objective To suppress the expression of CCR5 and CXCR4, the co-receptors for human immunodeficiency virus type 1(HIV-1), and thus inhibit HIV-1 from entering cells. Methods DNA fragments encoding either CCR5 or CXCR4 were amplified from healthy human peripheral blood mononuclear cells（PBMCs）by reverse transcript polymerase chain reaction（RT-PCR）and sequencing was performed. Correct fragments were inserted into Shuttle plasmid inversely, which was recombined with backbone plasmid containing homologous adenoviral genome in E.coli BJ5183. The recombinant plasmids were transfected into 293 cells in which they were packaged and amplified. Recombinant adenoviruses containing antisense RNA of CCR5 or CXCR4 were obtained and identified by RT-PCR, and the titres of them were determined by cytopathic effect (CPE) method. The U937 and MT4 cells were infected by recombinant adenoviruses containing antisense RNA of CCR5 (multiplicity of infection,MOI=100) and CXCR4(MOI=200), respectively. The expression of co-receptors on infected cell was measured by fluorescence activated cell sorter at 24, 48, 72 hours and 10 days after infection. In addition, the chemotactic activity and proliferation of infected cells were detected with Boyden chamber and 3H incorporation respectively. Results We constructed the recombinant plasmids and obtained the recombinant adenoviruses which contained antisense RNA of CCR5 or CXCR4 and were designated as pAd-antiR5 and pAd-antiX4 respectively. The titers of recombinant adenoviruses pAd-antiR5 and pAd-antiX4 were 5×1011 PFU/ml and 7×1010 PFU/ml, respectively. The expression rate of CCR5 on U937 cells decreased from 82.10% (blank control) to 1.12% (Ad-antiR5 infected), and that of CXCR4 on MT4 cells decreased from 42% (blank control) to 1.03% (Ad-antiX4 infected) 24 hours later. The expression rates of CCR5 on Ad-antiR5 infected U937 cells were 1.02%,1.26%,1.23% at 48 hours,72 hours,and 10 days later,respectively. The expression rates of CXCR4 on Ad-antiX4 infected MT4 cells were 1.13%,1.17%,1.22% at 48 hours,72 hours,and 10 days later,respectively. Moreover, the recombinant adenovirus had no effects on chemotactic activity and proliferation of the cells. Conclusion The recombinant adenovirus containing antisense CCR5 or CXCR4 can remarkably decrease the expression of co-receptors for HIV-1 on U937 or MT4 cells without affecting their chemotactic activities and proliferative abilities.

Key words recombinant adenovirus co-receptor antisense RNA inhibition

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