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论著

小鼠CD80和CD86基因RNA干扰慢病毒载体的构建及体外对树突状细胞的作用

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摘要

目的 构建小鼠CD80和CD86基因RNA干扰慢病毒表达载体，并在体外观察其对小鼠骨髓源性树突状细胞（dendritic cell，DC）的作用。方法 针对已经筛选确定的CD80基因RNA干扰有效靶序列，合成靶序列的双链DNA，接入pGCL-GFP载体，再与pHelper1.0和pHelper2.0质粒共转染293T细胞，包装产生慢病毒，以293T细胞GFP蛋白的表达水平测定病毒滴度；同法构建出CD86基因RNA干扰慢病毒载体。慢病毒感染体外培养的DC，通过荧光显微镜检测感染效率，Annexin V/PI双染色法检测感染细胞凋亡和坏死情况，流式细胞仪检测CD80和CD86的表达情况。结果 PCR和测序证实，pGCL-CD80shRNA和pGCL-CD86shRNA慢病毒载体构建正确，病毒滴度均达2×107TU/mL，适合感染DC的MOI值为20，此时慢病毒对DC具有低毒性，感染效率为85.42%。CD80和CD86表达的抑制率分别为82.05% 和77.78%。结论 成功构建出小鼠CD80和CD86基因RNA干扰慢病毒载体，其有明显抑制DC表面CD80和CD86的表达，这为移植物排斥提供了新的治疗手段。

关键词 慢病毒；CD80；CD86；RNA干扰；树突状细胞

分类号

Construction of lentiviral vectors targeting mouse CD80 and CD86 genes by RNA interference and their effects on dendritic cells in vitro

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Abstract

Objective To construct lentiviral vectors targeting mouse CD80 and CD86 genes by RNA interference and study their effects on bone marrow-derived dendritic cells（DC）in vitro. Methods The effective sequence of siRNA targeting CD80 gene was confirmed in our previous experiment. The complementary DNA containing both sense and antisense oligonucleotides of the targeting sequence was designed, synthesized. After being annealed, the double-stranded DNA was inserted into the pGCL-GFP vector. The resulting lentiviral vector was named pGCL-CD80shRNA. 293T cells were cotransfected with pGCL-CD80shRNA,pHelper1.0 and pHelper2.0. The titer of virus was tested according to the expression level of GFP. Lentiviral vector targeting mouse CD86 gene by RNA interference was constructed in the same way. The recombinant lentiviruses were used to infect DC separated from femurs and tibias of mice in vitro. The infection efficiency was assessed by fluorescence microscope. The cell viability of infected DC was determined by annexin V and propidium iodine staining. The expression of CD80 and CD86 was analyzed by flow cytometry. Results PCR and DNA sequencing demonstrated that pGCL-CD80shRNA and pGCL-CD86shRNA were constructed successfully. The titer of the recombinant lentiviruses was both 2×107TU/ml and the best MOI for lentivirus infecting DC was 20. Lentiviruses demonstrated a high (85.42%) infection efficiency of DC without affecting cellular viability. The inhibitory rates of CD80 and CD86 expression were 82.05% and 77.78% respectively. Conclusions Lentiviral vectors targeting mouse CD80 and CD86 genes by RNA interference were constructed successfully. The recombinant lentiviruses show significant inhibitory effects on CD80 and CD86 expression in DC. This approach is a potential therapeutic option for allograft rejection.

Key words lentivirus CD80 CD86 RNA interference dendritic cell

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