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基础研究

NOD小鼠CD8α+抗原基因克隆载体的构建及其 相关蛋白在树突状细胞中的表达

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摘要：

目的：构建NOD小鼠CD8α+抗原基因重组慢病毒载体，探讨其在树突状细胞（DCs）中的表达，阐明1型糖尿病（T1DM）的发病机理。方法：设计CD8α+基因特异性引物，从NOD小鼠胸腺淋巴细胞中提取总RNA，采用RT-PCR法扩增CD8α+基因，与质粒载体pCDF1-MCS2-EF1-COPGFP进行连接重组，经过转化、筛选、鉴定和序列测定后，应用Western blotting法检测CD8α+基因的表达。应用Nanofectin脂质体转染试剂将含有目的基因的质粒转染HEK293细胞，进行重组慢病毒载体包装，将包装后的重组慢病毒颗粒感染DCs，采用Western blotting法检测CD8α+蛋白的表达。结果：成功构建了NOD小鼠CD8α+抗原基因的重组质粒载体，重组表达载体经转染HEK293细胞获得了重组病毒的原液，重组病毒扩增后感染DCs，免疫荧光下可见DCs内含有绿色荧光蛋白(GFP)表达。结论：NOD小鼠CD8α+抗原基因重组病毒载体构建成功，且有相关蛋白表达。

关键词： 1型糖尿病；CD8α+ 抗原基因；慢病毒载体；基因克隆；树突状细胞

Construction of CD8α+ antigen gene clone vector and expression of its related protein in dendritic cells

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Abstract:

To construct the recombinant lentivirus vector of CD8α+ antigen gene and to discuss its protein expression in dendritic cells(DCs),and to clarify the pathogenesis of type 1 diabetes mellitus(T1DM). Methods The specific primer was designed and the full-length gene of CD8α+ antigen gene amplified from NOD mice CD8α+T lymphocytes were detected by RT-PCR.After plasmid transformation,clone selection,identification and DNA sequencing,the CD8α+ antigen gene were cloned into pCDF1-MCS2-EF1-COPGFP plasmid.The expression of CD8α+ antigen gene was analyzed by Western blotting method.The plasmid containing target gene was transfected into HEK293 cells with Nanofectin liposomes transfection reagent for restructuring virus carrier packing.After the DCs were transfected by the recombinant lentivirus carrier,the expression of CD8α+ protein was detected by Western blotting method.Results The recombinant plasmid vector of CD8α+ antigen gene of NOD mice was constructed successfully.After the HEK293 cells were transfected,the stock solution of recombinant virus was gotten.After the amplification of recombinant virus,the DCs were infected and the green fluorescent protein (GFP) was found in DCs under fluorescence microscope by immunofluorescence assay.Conclusion The recombinant virus carrier of CD8α+ antigen gene of NOD mice is constructed successfully and the related proteins can express in it.

Keywords: T1DM; CD8α+ antigen gene; lentiviral vector; gene cloning; dendritic cells

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