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论著

CD80/IgG融合基因真核表达载体的构建及在CHO细胞中的表达

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摘要

目的：构建CD80/IgG真核表达载体，使其在中国仓鼠卵巢细胞（CHO）中呈分泌型表达，为白血病免疫基因治疗的研究奠定基础。 方法： 采用多聚酶链反应（PCR）技术分别从小鼠脾细胞和含小鼠CD80全长互补DNA（cDNA） 的质粒pcDNA/B7中扩增出免疫球蛋白IgG1的Fc段和CD80胞外区，以定向克隆的方法将其串联至真核表达载体pcDNA3.0中，获得重组表达载体pcDNA/CD80-IgG；采用脂质体转染技术转染CHO细胞,G418筛选得到稳定表达细胞株；免疫印迹、斑点ELISA及流式细胞术鉴定融合蛋白的表达,并初步检测其活性。 结果： DNA测序证明两段基因正确克隆至pcDNA3.0的多克隆位点，CHO细胞的培养上清中可检测到融合蛋白的表达，其分子量大小和预期的基本一致，该融合蛋白可提高白血病细胞表面CD80的表达。 结论： 成功构建pcDNA / CD80-IgG真核表达载体，并在CHO细胞中分泌性表达有活性的CD80-IgG融合蛋白。

关键词 融合基因蛋白质类, CD80/IgG 免疫逃逸； 免疫疗法； 中国仓鼠卵巢细胞

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Construction of eukaryotic expression vector of fusion gene CD80-IgG and its expression in Chinese hamster ovary cells

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Abstract

<FONT face=Verdana>AIM: To construct the eukaryotic expression vector CD80-IgG by fusing the cDNA encoding extracellular portion of murine CD80 to the 5'-terminus of cDNA encoding Fc fragment of murine immunoglobulin G1 and to express the fusion protein in Chinese hamster ovary (CHO) cells. METHODS: The two cDNAs was amplified by PCR respectively from plasmid pcDNA/B7 containing the full-length cDNA of murine CD80 from murine spleen cells, and cloned to the eukaryotic expression vector pcDNA3.0 by directional cloning. The resultant recombinant plasmid pcDNA/CD80-IgG was transfected into CHO cells with liposome transfection reagent. The stably expressing cells were obtained by G418 screening. Western blot, Dot ELISA, and flow cytometry were used to detect the expression of the fusion protein and its immunological activity. RESULTS: DNA sequencing verified the correction of the construction of recombinant plasmid pcDNA/CD80-IgG. The expressed fusion protein was detected in the supernatant of transfected CHO cells and the molecular weight of the protein was similar to what we expected. Its immunological activity was also established. CONCLUSION: The recombinant plasmid pcDNA/CD80-IgG was successfully constructed and it expressed the fusion protein CD80-IgG.</FONT>

Key words Fusion proteins CD80-IgG Immune escape Immunotherapy； Chinese hamster ovary cells

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