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论著

人糖基磷脂酰肌醇(GPI)-B7-1真核表达载体的构建及表达

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摘要 目的：在仓鼠卵巢细胞CHO细胞中高效表达糖基磷脂酰肌醇GPI-B7-1(B7-1即CD80)融合蛋白，获得大量融合蛋白以研究GPI-B7-1在肿瘤治疗中的应用潜力。方法： 构建真核表达载体pc3.1/GPI-B7-1，利用脂质体lipofectamine 2000将其转染CHO细胞，G418加压筛选抗性克隆，流式细胞仪检测细胞膜上GPI-B7-1融合蛋白的表达情况，SDS-PAGE、Western blot分析鉴定其免疫活性。结果： 真核表达载体转染CHO细胞经G418筛选后，流式细胞分析证实为GPI锚定蛋白，SDS-PAGE在60 kD左右蛋白表达量明显高于对照组，在Western blot 在60 kD左右有一条强的棕色条带，说明GPI-B7-1在CHO中得到表达。结论： 在CHO细胞中高效表达GPI-B7-1融合蛋白，可获得大量融合蛋白，对进一步研究GPI-B7-1在肿瘤治疗中的应用打下基础。

关键词 糖基磷脂酰肌醇类； 基因,B7-1； CHO细胞； 真核表达； 印迹法,蛋白质

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Cloning and eukaryotic expressing of GPI-B7-1 in CHO

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Abstract

<FONT face=Verdana>AIM: To construct human GPI-B7-1 fusion protein and investigate the therapeutic potentials in the treatment of tumors. METHODS: The chimeric GPI anchored-B7-1 gene was obtained by overlap PCR and inserted into expressing vector pcDNA3.1, named pc3.1/GPI-B7-1. pc3.1/GPI-B7-1was transfected into CHO cells by lipofectamine 2 000 reagent. The CHO cells, expressing GPI-B7-1 on membranes, were obtained after selecting by G418. That was confirmed by flow cytometry, SDS-PAGE and Western blot. RESULTS: Recombinant vector pc3.1/GPI-B7-1 was successfully constructed and sequence result indicated that it was identical with reference sequence. The protein on transfected CHO cell membrane selected by G418 was confirmed to be GPI-anchored protein by flow cytometry, and GPI-B7-1 approximately 60 kD was conformed by SDS-PAGE and Western blot. CONCLUSION: A large amount of GPI-B7-1 fusion protein was obtained and will be further studied in the treatment of tumors.</FONT>

Key words Glycosylphosphatidylinositols Genes BT-1 CHO cells Eukaryotic expression Blotting Western

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