

## 肿瘤厌氧靶向双自杀基因治疗系统pTRKH2-PsT/CD、pTRKH2-PsT/UPRT的构建

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### Construction of Anaerobic Infant Bifidobacterial Mediated pTRKH2-PsT/CD and pTRKH2-PsT/UPRT Dual Suicide System for Tumor targeting Gene Therapy

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#### 摘要

目的 利用婴儿双歧杆菌对实体瘤低氧区的靶向效应, 构建肿瘤厌氧靶向双自杀基因治疗系统pTRKH2-PsT /CD和pTRKH2-PsT /UPRT。

方法 用PCR的方法从质粒pGEX/CD和pGEX/UPRT中扩增出CD基因和UPRT基因, 双酶切CD基因、UPRT基因和质粒pTRKH2-PsT, 分别连接后重组于大肠杆菌中。之后用电转化的方法将重组质粒转入婴儿双歧杆菌中。用RT-PCR检测该系统mRNA水平的表达, SDS-PAGE检测该系统在蛋白质水平的表达。在黑色素瘤B16-F10细胞上检测该系统的体外肿瘤细胞杀伤效果。

结果 成功地将CD基因和UPRT基因转入了质粒pTRKH2-PsT, CD基因和UPRT基因的测序结果表明序列与Genebank公布的序列一致。RT-PCR检测到CD和UPRT mRNA水平的明显表达。在含有CD基因的婴儿双歧杆菌细胞全蛋白中发现了CD蛋白质的表达, 在含有UPRT基因的婴儿双歧杆菌上清液中发现了UPRT蛋白质的表达。黑色素瘤细胞的低存活率证明了pTRKH2-PsT/CD、pTRKH2-PsT/UPRT自杀基因治疗系统对黑色素瘤的显著杀伤作用。

结论 肿瘤厌氧靶向双自杀基因治疗系统pTRKH2-PsT/CD、pTRKH2-PsT/UPRT构建成功并显示出杀伤肿瘤细胞的作用。

关键词: 婴儿双歧杆菌 自杀基因 CD基因 UPRT基因 肿瘤靶向性

#### Abstract:

Objective To construct pTRKH2-PsT/CD and pTRKH2 PsT/UPRT dual suicide gene therapy system into tumor-hypoxia-targeting carrier of Infant Bifidobacterium.

Methods CD gene and UPRT gene were amplified from pGEX/CD and pGEX/UPRT by PCR. The CD gene, UPRT gene were inserted into lactic acid expressing plasmid pTRKH2-PsT, then constructed two recombinant plasmids of pTRKH2-PsT/CD and pTRKH2-PsT/UPRT were introduced into Bifidobacterium using electroporation. The expression of CD and UPRT in Bifidobacterium were detected by RT-PCR or through SDS-PAGE and Silver staining. The killing effects of pTRKH2-PsT/CD and pTRKH2-PsT/UPRT suicide gene therapy system on Melanoma B16 F10 cells were measured using methyl thiazolyl tetrazolium (MTT) assay.

Results The CD gene and UPRT gene was successfully recombined into plasmid pTRKH2 -PsT. The sequencing results of CD gene and UPRT gene in dual suicide system are consistent with data in Genebank. Both mRNA and protein level of CD and UPRT were effectively expressed in gene recombinant Infant Bifidobacterium. The survival rate of Melanoma B16 F10 cells treated with CD/UPRT and 5-FC ranged from 1.7%~13.3%, while the rates in

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control, CD and UPRT groups ranged from 81.3%~91.7%,26.7%~58.3% and 78.3%~86.7%, respectively.

Conclusion CD gene and UPRT gene were successfully inserted into pTRKH2-PsT vector and effectively recombined into Infant Bifidobacterium. This dual suicide gene therapy system showed a powerful killing efficiency on tumor cells.

Key words: Bifidobacterium Infantis Suicide gene CD gene UPRT gene Tumor targeting

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