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基础研究

hTERT启动子调控的融合自杀基因CD:UPRT载体的构建及其应用

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Construction of Cytosine Deaminase:Uracil Phosphoribosyltransferase/5-fluorocytosine Gene Therapy System under Control of Human Telomerase Reverse Transcriptase Promoter and Its Application

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全文: PDF (342 KB) HTML (0 KB) 输出: BibTeX | EndNote (RIS) 背景资料

摘要 目的 构建hTERT启动子调控的融合自杀基因CD:UPRT表达载体，研究其对人胃癌细胞SGC7901的体外靶向性杀伤作用。方法 PCR扩增hTERT核心启动子片段，克隆入荧光素酶报告基因质粒pGL3 Basic，检测hTERT启动子在人胃癌细胞SGC7901和人成纤维细胞HLF中的转录活性。构建hTERT启动子调控的CD:UPRT基因表达载体hTERT CD:UPRT，将其和CMV启动子调控的CD:UPRT基因表达载体pcDNA3.1 CD:UPRT用脂质体转染法分别转染入SGC7901和HLF细胞，筛选稳定表达细胞系，用RT PCR和Western blot方法检测CD基因的表达，用MTT法检测5 FC对转染细胞的杀伤作用。结果 成功克隆hTERT核心启动子；荧光素酶活性检测显示，hTERT启动子在SGC7901细胞中的转录活性为阳性对照的(21.50±2.15)%，而在HLF细胞中仅有背景活性。成功构建hTERT启动子调控的CD:UPRT基因表达载体，转染pcDNA3.1 CD:UPRT的SGC7901和HLF细胞以及转染hTERT CD:UPRT的SGC7901细胞在mRNA和蛋白质水平均可检测到CD基因的表达，且对5 FC敏感；而转染hTERT CD:UPRT的HLF细胞未检测到CD基因的表达，对5 FC不敏感。结论 构建的hTERT启动子调控的融合自杀基因系统CD:UPRT/5 FC能在体外靶向性杀伤SGC7901细胞。

关键词： 人端粒酶逆转录酶启动子 胞嘧啶脱氨酶:尿嘧啶磷酸核糖转移酶 胃癌 靶向基因治疗中图分类号:R73054

Abstract: :Objective To const ruct the expression vector containing CD :UPRT(cytosine deaminase :uracil phosphoribosylt ransferase) genes under the cont rol of the hTERT promoter and investigate it s specific killing effect s on human gast ric cancer cells SGC7901 in vi t ro. Methods The hTERT promoter was PCR amplified and cloned into the p GL32Basic vector. The recombinant was t ransfected into SGC7901 cells and normal human fibroblast cells HL F to detect the t ranscriptional activities of the hTERT gene promot2 er. The expression vector containing CD :UPRT genes under the cont rol of the hTERT promoter named as hTERT2CD :UPRT was const ructed. This vector and the vector containing CD :UPRT genes under the cont rol of cytomegalovirus (CMV) promoter named as pcDNA3. 12CD : UPRT were t ransfected into SGC7901 and HL F cells , respectively. The t ransfected cells were selected by G418. The expression of the CD gene was detected by RT2PCR and Western blot . MTT analysis was used to determine the cyto2 toxic effect s of the CD :UPRT/ 52FC system. Results The hTERT promoter was PCR amplified success2 fully. Luciferase assay showed the relative luciferase activity of SGC7901 by the hTERT promoter was (21. 50 ±2. 15) % and that of HL F cells was only (0. 40 ±0. 07) %. The expression vector hTERT2CD : UPRT was successfully const ructed. Af ter stably t ransfected with pcDNA3. 12CD :UPRT , SGC7901 and HL F cells both expressed CD genes and were sensitive to 52FC , while positive only in SGC7901 cells af2 ter stably t ransfected with pcDNA 3. 12CD :UPRT. Conclusion The hTERT promoter can specifically cont rol the CD :UPRT gene expression in SGC7901 cells but not in the normal cells and the CD :UPRT/ 52FC system under cont rol of the hTERT promoter can specially kill SGC7901 cells in vi t ro.

Key words: hTERT promoter Cytosine deaminase:uracil phosphoribosyltransferase Gastric cancer Targeted gene therapy

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