

hTERT启动子的克隆及hTERT启动子/SV40增强子在食管癌细胞中的转录活性

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Cloning of hTERT Promoter and Transcriptional Activity of hTERT Promoter/SV40 Enhancer in Esophageal Cancer Cells

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全文: PDF (713 KB) HTML (0 KB) 输出: BibTeX | EndNote (RIS) 背景资料

摘要 目的 克隆hTERT启动子核心序列, 研究hTERT启动子/SV40增强子在食管癌细胞中的联合转录活性。 方法 以人基因组DNA为模板, PCR扩增hTERT启动子核心片段; 将其分别插入荧光素酶基因报告质粒pGL3-Basic和pGL3-Enhancer中, 构建hTERT启动子调控的表达载体pGL3-hTERTp和由hTERT启动子/SV40增强子联合调控的表达载体pGL3-hTERTp-SV40en, 将上述重组质粒分别瞬时转染食管癌细胞Eca-109、EC1和人胚肺成纤维细胞MRC-5, 用荧光素酶检测试剂盒检测转染细胞中荧光素酶基因的表达式水平并以此计算hTERT启动子和hTERT启动子/SV40增强子在各种细胞中的转录活性。结果 克隆出长213 bp的hTERT启动子核心片段, DNA测序结果与GenBank中hTERT启动子的碱基序列完全一致; 成功构建真核表达载体pGL3-hTERTp和pGL3-hTERTp-SV40en; hTERT启动子在食管癌细胞Eca-109和EC1中均有转录活性, 在MRC-5细胞中无明显转录活性; hTERT启动子/SV40增强子在食管癌细胞Eca-109和EC1中的转录活性显著高于hTERT启动子的单独转录活性。结论 hTERT启动子在食管癌细胞中具有靶向性转录活性, SV40增强子能显著增强hTERT启动子在食管癌细胞中的转录活性, 有可能作为肿瘤靶向性基因治疗的转录调控元件。

关键词: hTERT 启动子 SV40增强子 食管癌 靶向性表达

Abstract: Objective To clone core sequence of hTERT promoter, and study transcriptional activity of hTERT promoter/SV40 enhancer in esophageal cancer cell. Methods hTERT promoter was amplified from human genomic DNA using polymerase chain reaction (PCR); hTERT promoter was inserted into pGL3-Basic and pGL3-enhancer to construct luciferase gene expression driven by hTERT promoter (named as pGL3-hTERTp) or by hTERT promoter/SV40 enhancer (named as pGL3-hTERTp-SV40en), respectively. The recombinants were transiently transfected into esophageal cancer cells of Eca-109, EC1 and human embryo lung fibroblast MRC-5, respectively, then expression level of luciferase gene in transfected cells was studied to evaluate transcriptional activities of hTERT promoter and hTERT promoter/SV40 enhancer in esophageal cancer cells. Results A 213 bp core-sequence of hTERT promoter was cloned successfully, and DNA sequencing showed its sequence the same as that registered in GenBank; recombinants of pGL3-hTERTp and pGL3-hTERTp-SV40en were successfully constructed. hTERT promoter had transcriptional activity in esophageal cancer cells while no transcriptional activity in MRC-5 cells; The transcriptional level of hTERT promoter/SV40 enhancer in esophageal cancer cells was significantly higher than hTERT promoter alone. Conclusion hTERT promoter has selective transcriptional activity in esophageal cancer cells; SV40 enhancer could significantly elevate transcriptional level of hTERT promoter, and it could play an important role in targeting gene therapy for tumor.

Key words: hTERT Promoter SV40 enhancer Esophageal cancer Targeting expression

收稿日期: 2009-08-13;

引用本文:

唐小军, 戴天阳, 廖斌等. hTERT启动子的克隆及hTERT启动子/SV40增强子在食管癌细胞中的转录活性[J]. 肿瘤防治研究, 2010, 37(11): 1230-1233.

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鄂ICP备08002248号

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