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论著

重组Ad-Cp-CDglyTK双自杀基因腺病毒载体构建及体外抑制鼻咽癌细胞的实验研究

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摘要 目的：
构建含EB病毒Cp启动子的CDglyTK双自杀基因靶向腺病毒载体 Ad-Cp-CDglyTK，探讨Cp启动子能否特异性调控自杀基因在转染鼻咽癌细胞中表达及靶向性治疗鼻咽癌的可行性。方法：采用pDC316穿梭质粒系统，高保真PCR扩增tk、 cd、 Cp等基因序列，采用定向克隆方法构建含Cp启动子的双自杀基因pDC316-CP-CDglyTK重组质粒，经DNA测序、酶切法鉴定所构建质粒，在293细胞中进行重组腺病毒Ad-Cp-CDglyTK包装、扩增、纯化与病毒滴度测定，体外转染鼻咽癌细胞株CNE1与正常鼻咽NP69细胞株，RT-PCR法检测转染细胞中CDglyTK基因的表达，MTT法观察Ad-Cp-CDglyTK/GCV+5-FC系统对CNE1细胞株的体外杀伤作用。结果： 经DNA测序、限制性酶切法分析显示pDC316-Cp-CDglyTK含完整正确的tk、cd、Cp基因序列，在293细胞中包装扩增后病毒滴度为5.6×1012 TCID50/L，体外转染鼻咽癌CNE1细胞株与正常鼻咽NP69细胞株后，采用RT-PCR法从CNE1细胞株总RNA中扩出Cp片段，而NP69细胞株未检测到相应基因mRNA表达，MTT结果显示经前体药物处理转染后CNE1细胞株与NP69细胞株，5-FC+GCV联合用药较单一前体药物对CNE1细胞具有更强的抑制作用（P＜0.05），联合用药对NP69细胞株未见明显杀伤作用。结论： Cp启动子可以特异性调控CDglyTK融合自杀基因在鼻咽癌细胞株(CNE1)中表达，融合自杀基因/前药系统较单一基因对鼻咽癌细胞具有更强的杀伤作用。

关键词 鼻咽肿瘤； Cp启动子； 腺病毒,人； 基因疗法

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Antitumor effects of recombinant vectors carrying CDglyTK suicide gene on nasopharyngeal carcinoma cell in vitro

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Abstract

<FONT face=Verdana>AIM:To construct the recombinant adenovirus carrying fusion suicide gene CDglyTK with the C promoter(Cp), one of the key factors in controlling Epstein-Barr virus latent gene expression, and to investigate if the Cp mediates the expression of CDglyTK in CNE1 cells and kills the cancer cells specifically.
METHODS:The tk, cd, Cp sequences were amplified by PCR and subcloned into corresponding sites of pDC316 vector with directional cloning method to construct the pDC316-CP-CDglyTK. The plasmid was analyzed by DNA sequencing and enzyme digestive method. The recombinant adenovirus of Ad-Cp-CDglyTK was packaged, amplified and purified in 293 cells, and the virus titre was determined by TCID50 method. The CDglyTK gene expression in CNE1 and NP69 were examined by reverse transcription-polymerase chain reaction (RT-PCR) after in vitro transfection in CNE1 and NP69 cells. The killing effect of Ad-Cp-CDglyTK/GCV+5-FC on CNE1 cells was
detected by MTT method.
RESULTS:The results of restriction enzyme digestion and DNA sequencing showed that the tk, cd, and Cp gene were inserted into the pDC316 plasmid in correct orientations. The titer of the recombinant adenovirus was 5.6×1012 TCID 50/L. The Cp fragment was amplified from the total RNA of the transfected CNE1 cells by RT-PCR. The mRNA of CDglyTK

gene expression was not detected in NP69 cells. The MTT results showed that after administration of GCV and 5-FC, the killing effects of fusion gene were much better than those of single gene therapy.
CONCLUSION:The C promoter specifically mediates the expression of CDglyTK in CNE1 cells. The Ad-Cp-CDglyTK/GCV+5-FC has much better killing effects on CNE1 cells than single gene. </FONT>

Key words Nasopharyngeal neoplasms C promoter Adenoviruses human Gene therapy

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