中国病理生理杂志 2009 24 (6): 1111-1116 ISSN: 1000-4718 CN: 44-1187/R

论著

AFP启动子驱动的yCD/TK双自杀基因靶向杀伤肝癌细胞的体内外研究

华赟鹏[1],李宝金[2],汪勇[1],王瑛[3],朱宝益[4]

中山大学附属一院1肝胆外科，3超声科，4中山大学中山医学院2004级临床医疗系，广东 广州 510080；2北京大学深圳医院肝胆外科，广东 深圳 518036

收稿日期 2008-12-2 修回日期 2009-3-17 网络版发布日期 2009-8-16 接受日期 2009-3-17

摘要 目的： 研究携带甲胎蛋白（AFP）启动子的酵母菌胞嘧啶脱氨酶/胸苷激酶（yCDglyTK）双自杀基因体内外靶向性杀伤肝癌细胞的效果和机制。方法: 构建携带AFP启动子的yCD/TK双自杀基因表达质粒。通过阳离子脂质体将携带AFP启动子的yCD/TK双自杀基因转染HepG2和SMMC7721细胞，用MTT法测定不同浓度氟胞嘧啶（5-FC）、更昔洛韦（GCV）及联合治疗的杀伤作用，用流式细胞仪检测细胞周期。建立裸鼠肝癌皮下种植瘤模型，观察自杀基因体内杀瘤效果以及细胞凋亡的情况。结果: 成功构建的携带AFP启动子的yCD/TK双自杀基因靶向性地在AFP阳性的HepG2细胞上表达，而AFP阴性的SMMC7721细胞无表达，GCV、5-FC及两者联合可有效抑制HepG2细胞生长，随药物浓度的增高而杀伤作用增强，药物间抑瘤效果比较是GCV＋5-FC＞5-FC＞GCV，而SMMC7721细胞的生长未受影响。体内实验可见GCV、5-FC及两药联合对转染后的HepG2细胞种植瘤有明显的抑制效果，并检测到明显的细胞凋亡，而对SMMC7721细胞种植瘤的生长无影响，种植瘤内极少凋亡细胞。结论: 携带AFP启动子的yCD/TK双自杀基因能有效地靶向性地杀伤AFP阳性的肝癌细胞，细胞凋亡可能是其杀伤的重要机制之一。

关键词 癌,肝细胞 基因疗法 自杀基因

分类号 R735.7

Treatment of hepatocellular carcinoma in vitro and in vivo with yCD/TK double suicide gene driven by AFP promoter

HUA Yun-peng[1],LI Bao-jin[2],WANG Yong[1],WANG Ying[3],ZHU Bao-yi[4]

1Department of Hepatobiliary Surgery,3Department of Ultrasound, The First Affiliated Hospital, Sun Yat-sen University; 4Grade 2004 of Medical College, Sun Yat-sen University, Guangzhou 510080, China; 2Department of Hepatobiliary Surgery, Peking University Shenzhen Hospital, Shenzhen 518036, China. E-mail: hyp0427@163.com

Abstract

<FONT face=Verdana>AIM: To study the effect and mechanism of yeast cytosine deaminase/ thymidine kinase (yCD/TK) double suicide gene driven by alpha fetoprotein (AFP) promoter on hepatocellular carcinoma (HCC) in vitro and in vivo. METHODS: The expression plasmid with yCD/TK double suicide gene, which was driven by AFP promoter, was constructed. HepG2 (AFP positive) and SMMC7721 (AFP negative) human HCC cell lines were both transfected with the above-mentioned expression plasmid through cationic liposome. The cells were treated with 5-fluorocytosine (5-FC) and/or ganciclovir (GCV) at different concentrations. The cell proliferation and cell cycle phase were evaluated by MTT test and flow cytometry respectively. The effect of double suicide gene on HCC xenografts in nude mice was observed through measuring the tumor size and the number of apoptosis cells. RESULTS: The double suicide gene was expressed selectively on HepG2 cells, rather than on SMMC7721 cells. The 5-FC and/or GCV inhibited effectively the proliferation of HepG2 cells in a dose-dependent manner, but had no influence on SMMC7721 cells. The inhibitory effect on HepG2 cells among different treatments was GCV+5-FC>5-FC>GCV. In vivo, the treatments inhibited markedly the growth of HepG2 cell xenografts in nude mice, transfected with yCD/TK gene. More apoptotic cells were found in HepG2 xenografts after the treatment. However, the growth of SMMC7721 cell xenografts could not be inhibited by this double suicide gene therapy, and few apoptotic cells were found. CONCLUSION: yCD/TK double suicide gene driven by AFP promoter has a significant efficacy in treatment of AFP positive HCC. Cell apoptosis may be an important mechanism of yCD/TK

double suicide gene-inhibiting the growth of HCC.</FONT>

Key words Carcinoma hepatocellular Gene therapy Suicide gene

DOI: 1000-4718

通讯作者 华赟鹏 hyp0427@163.com