吉林大学学报(医学版) 2010, 36(1) 14-19 DOI: ISSN: 1671—587X CN: 22—1342/R

基础研究

HRE增强hTERT启动子-tk/GCV基因系统对缺氧环境肿瘤细胞的杀伤作用

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摘要：

目的：探讨缺氧反应元件(HRE)增强hTERT启动子-tk/GCV逆转录病毒基因系统对缺氧环境肿瘤细胞的体外杀伤作用。 方法：构建由HRE缺氧反应元件修饰的hTERT杂合启动子和hTERT单一启动子引导单纯疱疹病毒胸苷激酶(HSV-tk)基因表达的重组逆转录病毒载体，在脂质体介导下分别转染PA317包装细胞，G418筛选，直至出现抗性克隆。将重组病毒感染人肺癌细胞SPC-A1和人肝癌细胞Bel-7402，G418筛选出抗性克隆肿瘤细胞。实验分为对照组、SPC-A1/tk组和Bel-7402/tk组。在缺氧处理条件下，MTT法检测前体药物丙氧鸟苷(GCV)对SPC-A1/tk和Bel-7402/tk细胞的杀伤效应，流式细胞仪检测其细胞凋亡率和细胞周期的变化。 结果:与对照组比较,缺氧环境培养的SPC-A1/tk和Bel-7402/tk细胞对GCV的敏感性明显增强，其中细胞凋亡率明显升高（P<0.001），细胞周期各期细胞数有所减少（P<0.05）。与hTERT启动子引导的tk基因系统组细胞比较，［HRE］hTERT杂合启动子引导的tk基因系统组肿瘤细胞存活率更低（P<0.01），诱导肿瘤细胞凋亡率明显升高（P<0.01），G_1期和G_0期细胞数有所增高（P<0.05），S期细胞数减少（P<0.05）。结论：HRE能增强hTERT启动子-tk/GCV自杀基因系统对缺氧环境中肿瘤细胞的体外杀伤作用。

关键词： 肿瘤；hTERT启动子；缺氧反应元件； HSV-tk基因；缺氧

Enhancement of |HRE on killing effect of |hTERT promoter-tk/GCV suicide gene therapy system on tumor cells under hypoxic condition

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Abstract:

Abstract:Objective
To investigate the enhancement of hypoxia responsive element (HRE) on killing effect of retrovirus mediated HSV-tk/gancyclovir (GCV) suicide gene system controlled by human telomerase reverse transcriptase (hTERT) promoter on tumor cells under hypoxia condition.Methods The recombinant retroviral vectors including the HRE hypoxia responsive element,the hTERT promoter and suicide gene tk based on retroviral vector pLNCL were constructed. The recombinant retroviral vector was transfected into PA317 cells for package by liposome transfection， 2 weeks after G418 selection，G418-resistant PA317 colonies were obtained and amplified. The supernatant of cell culture was harvested and used to infect NIH3T3 cells to measure the viral titer of recombinant retrovirus.The viral supernatant was used to infect lung cancer cells SPC-A1 and liver cancer cells Bel-7402.The positive clones were obtained by G418 selection.The experiment was divided into control group,SPC-A1/tk group and Bel-7402/tk group.Under the hypoxia (1%O2 ， 94%N2) condition,GCV-mediated cell growth inhibition was determined with method of tetrazolium(MTT).FCM combined with PI and AnnexinV-FITC double pigmention methods were used to observe the apoptosis and cell cycle of two tumor cell lines infected by the recombinant retrovirus.
Results As compared with control group, SPC-A1/tk and Bel-7402/tk showed high sensitivity to GCV in vitro under hypoxia condition,the apoptotic rate was increased significantly (P<0.001).As compared with group of recombinant retrovirus with tk driven by the hTERT promoter,the survival rate in group of recombinant retrovirus with tk driven by HRE-modified hTERT promoter was decreased significantly (P<0.01),the apoptotic rate was increased significantly (P<0.01),and both the cell populations at G_1 and G_0 phases were increased significantly (P<0.05),and the cell population at S phases was decreased significantly (P<0.05).Conclusion HRE can enhance the killing effect of HSV-tk/GCV suicide gene system controlled by the hTERT promoter on tumor cells line under hypoxia condition.

Keywords:

收稿日期 2009-08-16 **修回日期** **网络版发布日期** 2010-01-28

DOI:

基金项目:

国家自然科学基金资助课题 （30471284）;吉林省教育厅“十一五”科学技术研究项目资助课题(吉科合字［2008］第73号)；吉林农业大学科学研究启动基金资助课题（200705）

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