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hMAM-EP 调控 HSV-TK 腺病毒载体的构建及其对乳腺癌细胞的靶向杀伤

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摘要：

目的：分别构建人乳腺珠蛋白(human mammaglobin, hMAM）基因增强子和启动子（enhancer and promoter of hMAM, hMAM-EP）调控的增强型绿色荧光蛋白（enhanced green fluorescent protein, EGFP）报告基因和单纯疱疹病毒胸苷激酶herpes simple virus ymidine kinase, HSV-TK）自杀基因两种重组腺病毒载体，探讨 hMAM-EP 调控的 HSV-TK 在乳腺癌细胞特异性表达及其对乳腺癌的靶向治疗作用。 方法： 构建hMAM-EP-EGFP和hMAM-EP-TK重组质粒载体，将重组质粒目的基因转移到腺病毒骨架黏粒载体pAxcwit2，并转染HEK 293细胞获得重组腺病毒载体Ad-EP-EGFP和Ad-EP-TK。将Ad-EP-EGFP感染乳腺癌细胞T-47D、ZR-75-30和鼻咽癌细胞5-8F，荧光显微镜观察EGFP的表达。将Ad-EP-TK感染T-47D细胞，给予1、10、20、50 μg/ml前药更昔洛韦（ganciclovir, GCV），观察 TK基因对乳腺癌细胞的特异杀伤作用。 结果： 成功构建 hMAM-EP 调控的重组腺病毒载体Ad-EP-EGFP和Ad-EP-TK。Ad-EP-EGFP感染后，乳腺癌T-47D细胞可见EGFP表达，但ZR-75-30细胞和 5-8F 细胞无表达。与未感染组或感染Ad-EP-EGFP组相比，Ad-EP-TK重组腺病毒联合GCV（50 μg/ml）组T-47D细胞存活率显著降低\[（35 69±0.07)% vs (91.74±0.02)%，(87.69±0.11)%； P <0.05\]，且随GCV质量浓度的加，T-47D细胞存活率逐渐下降，在MOI=100、GCV质量浓度分别为1、10、20、50 μg/ml条件下，细胞存活率分别为(94.34±0.04)%、(86.26± 0 0 2)%、(66.51±0.09)%、(35.69.07)%。 结论： hMAM-EP 调控的 HSV-TK 自杀基因在乳腺癌T-47D细胞中特异性表达，Ad-EP-TK 联合GCV可靶向杀伤乳腺癌T-47D细胞。

关键词：乳腺癌 人乳腺珠蛋白基因 单纯疱疹病毒胸苷激酶基因 更昔洛韦 重组腺病毒 基因治疗

Construction of recombinant adenovirus carrying HSV-TK controlled by hMAM enhancer and promoter and its targeted killing effect on human breast cancer cells

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Abstract:

Objective : To construct two recombinant adenovirus vectors carrying reporter gene enhanced green fluorescent protein (EGFP) or suicide gene herpes simple virus thymidine kinase (HSV-TK) at the downstream of enhancer and promoter of human mammaglobin (hMAM-EP). To explore breast-cancer-cell-specific regulation effect of hMAM-EP and new methods of targeted therapy for breast cancer. Methods: Two recombinant plasmid vectors, hMAM-EP-EGFP and hMAM-EP-TK, were constructed, which respectively carried reporter gene EGFP and suicide gene HSV-TK at the downstream of hMAM-EP. Recombinant adenovirus vectors Ad-EP-EGFP and Ad-EP-TK were obtained after the target genes from the recombinant plasmids were transferred into adenovirus skeleton cosmid pAxcwit2; recombinant adenovirus vectors Ad-EP-EGFP and Ad-EP-TK were then transfected into breast cancer T-47D cells, ZR-75-30 cells and nasopharyngeal cancer 5-8F cells. The expression of EGFP was observed under a fluorescence microscope. Recombinant adenovirus Ad-EP-TK-infected T-47D cells were cultured with 1, 10, 20 and 50 μg/ml prodrug GCV to observe specific cell-killing effect on breast cancer cells. Results: The recombinant plasmid vectors Ad-EP-EGFP and Ad-EP-TK controlled by hMAM-EP were successfully constructed. EGFP could be observed in human breast cancer T-47D cells infected with Ad-EP-EGFP recombinant adenovirus, and could not be detected in ZR-75-30 and 5-8F cells. Compared with un-infected and Ad-EP-EGFP-infected groups, the survival rate of T-47D cells in Ad-EP-EGFP-infection combined with GCV (50 μg/ml) group was significantly decreased (\[35.69±0.07\]% vs \[91.74±0.02\]%, \[87.69±0 11\]%, P <0.05). With an increase in mass concentration of GCV, the survival rate decreased. Cell survival rates were (94.34±0.04)%, (86.26± 0.02)%, (66.51±0.09)% and (35.69±0.07)% when T-47D cells were infected with hMAM-EP-TK in a MOI of 100 and cultured with 1, 10, 20, and 50 μg/ml GCV. Conclusion: HSV-TK suicide gene controlled by hMAM-EP is specifically expressed in breast cancer T-47D cells, and T-47D cells can be killed by Ad-EP-TK combined with GCV.

Keywords:breast cancer human mammaglobin gene herpes simple virus thymidine kinase gene ganciclorir recombinant adenovirus gene therapy