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腺病毒介导RNA干扰抑制血管内皮生长因子的表达治疗人肺腺癌的实验研究

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黄盛东 李白翎 龚德军 袁扬 刘晓红 张冠鑫 徐志云

第二军医大学长海医院胸心外科,上海,200433

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摘要：

目的:构建针对人血管内皮生长因子(VEGF)的腺病毒载体pAd-Easy/VEGF,应用RNA干扰的方法,观察其在体内和体外对人肺腺癌细胞株A549生长的抑制作用.方法:应用PCR构建RNA干扰pAd-Easy/VEGF腺病毒载体,并利用该线性化质粒用Lipofectamine 2000转染293细胞,制备携带人VEGF的腺病毒转染A549细胞.荧光显微镜和流式细胞仪观察增强型绿色荧光蛋白(EGFP)的转染效率,RT-PCR和蛋白质印迹法检测VEGF mRNA的表达,MTT比色法测定活细胞数并绘制细胞生长曲线.同时制备裸鼠A549细胞移植瘤模型,观察肿瘤生长情况.结果:pAd-Easy/VEGF重组质粒经测序证实已把预设人VEGF基因RNA干扰的siRNA模板序列插入载体.转染重组腺病毒及空病毒24 h后流式细胞术测得转染效率分别为100％、99.7％.pAd-Easy/VEGF组VEGF表达水平较生理盐水对照组明显降低.pAd-Easy/VEGF组细胞生长明显减缓.pAd-Easy/VEGF治疗组肿瘤体积和质量明显小于对照组(P＜0.01).结论: pAd-Easy/VEGF介导的VEGF shRNA能有效抑制A549细胞中VEGF的表达,并在体内抑制肿瘤生长.

关键词：RNA干扰 血管内皮生长因子 腺病毒科 肺肿瘤 腺癌

Suppression of vascular endothelial growth factor expression by adenovirus-mediated RNA interference in treatment of lung adenocarcinoma cells

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HUANG Sheng-dong LI Bai-ling GONG De-jun YUAN Yang LIU Xiao-hong ZHANG Guan-xin XU Zhi-yun

Fund Project:Supported by the Key Program of Shanghai Science and Technology Committee （04JC14006）.

Abstract:

Objective: To investigate the inhibitory effects of RNA silencing via adenovirus-mediated vascular endothelial growth factor（VEGF） shRNA on proliferation of lung adenocarcinoma ceils in vitro and in vivo. Methods: PCR method was used to construct a pAd-Easy/VEGF adenovirus vector containing enhanced green fluorescent protein （EGFP） gene and expressing VEGF shRNA. The 293 ceils were transfected with the linearized pAd-Easy/VEGF using Lipofectamine2000. Then lung adenocarcinoma cells A459 were transfected with the constructed vector. The EGFP expression was detected by fluorescent microscopy and flow cytometry. VEGF mRNA expression was examined by RT-PCR and Western blotting. The cell growth was observed with MTT method and the growth curve was plotted. Meanwhile, nude mice were transplanted with A549 cells to establish tumor models and the growth of tumors were observed. Results: The recombinant pAd-Easy carrying shRNA targeting VEGF had been constructed and the aim sequence had been obtained. The transfection efficiencies in pAd-Easy/VEGF and blank vector transfected A549 cells were 100% and 99.7%, respectively. RT-PCR and Western blotting showed a remarkable decrease of VEGF expression in the pAd-Easy/VEGF group compared with normal saline group. The tumor growth in pad-Easy/VEGF group was obviously slowed down and the weight and volume of tumors were both significantly lower than those of the control group （all P〈0. 01）. Conclusion: The shRNA targeting VEGF constructed in the present study can efficiently decrease the VEGF expression in A549 cells in vitro and suppress the growth of A549 ceils in vivo.

Keywords:RNA interference vascular endothelial growth factor adenoviridae lung neoplasms adenocarcinoma

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