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论著

靶向腺病毒载体介导的EGFP基因在甲胎蛋白阳性肝癌细胞的特异表达

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摘要 目的：建立一种在甲胎蛋白(AFP)阳性的肝癌细胞中靶向表达目的基因的重组腺病毒载体。方法： 基于腺病毒载体Adeno-XTM Expression system,以300 bp AFP特异启动子替换穿梭质粒Pshuttle中CMV启动子，将增强型绿色荧光蛋白(EGFP)基因作为报告基因亚克隆至Pshuttle，HEK293细胞包装腺病毒，收集病毒后分别转染人正常肝脏LO2细胞，人肝癌HepG2细胞及HeLa细胞；通过Northern杂交检测EGFP基因在3种细胞中的转录水平，荧光显微镜下观察3种细胞中绿色荧光蛋白的表达。结果：Northern杂交显示，HepG2细胞中有大量EGFP基因的转录，而正常肝细胞LO2和HeLa细胞中仅能检测到微量基因的转录；荧光显微镜检测发现HepG2细胞内有强绿色荧光表达，而在LO2以及HeLa细胞内见极弱绿色荧光。 结论： 在AFP特异启动子作用下，腺病毒携带的目的基因在AFP阳性的肝癌细胞中得到显著转录和表达，而在非AFP阳性细胞仅微量转录，蛋白表达极弱。该腺病毒载体可作为AFP阳性的肝癌基因靶向治疗的适宜载体。

关键词 甲胎蛋白类； 启动区(遗传学)； 腺病毒载体； HepG2细胞

分类号 R363

Construction of recombinant adenovirus vector expressing EGFP gene specifically in AFP producing liver cancer cells

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Abstract

<FONT face=Verdana>AIM: To construct a recombinant adenovirus vector carrying AFP promoter to specifically express a targeting gene in hepatocellular carcinoma HepG2 cells. METHODS: Based on the Adeno-XTM Expression system, the CMV promoter was replaced by a 300 bp a-fetoprotein promoter. The EGFP(enhanced green fluorescent protein) gene as a report gene was inserted to the multiple-cloning site(MCS). The normal liver LO2 cells, hepatocellular carcinoma HepG2 cells and HeLa cells were infected by the recombinant adenovirus, respectively. Northern blotting and fluorescence microscope were used to detect the transcription level of EGFP gene and its protein expression, respectively. RESULTS: Northern blotting showed that the target gene was markedly transcribed in HepG2 cells, but slightly in LO2 and HeLa cells. Under the fluorescence microscope, strong EGFP expression was seen in HepG2 cells but very weakly in HeLa and LO2 cells. CONCLUSION: Under the control of the 300 bp human AFP promoter, the target gene carried by the recombinant adenovirus was expressed in the AFP-producing HepG2 cells at a very high level, but not or very weakly in AFP negative cells. This adenovirus system can be used as a new, potent and specific approach for the gene-targeting therapy for the AFP producing primary hepatoma.</FONT>

Key words Alpha-fetoproteins Promoter regions(genetics) Adenovirus vector HepG2 cells

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