中国生物工程杂志 2012, 32(01) 15-20 DOI: ISSN: 1671-8135 CN: 11-4816/Q

论文

携带不同数量增强子的嵌合型肝脏特异性启动子的构建及其体外实验研究

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摘要:

通过PCR手段成功获得肝细胞特异性启动子人α1-抗胰蛋白酶启动子hAATp(human α1-antitrypsin promoter,hAATp)及具有增强子功能的肝脏特异的肝调控区HCR (hepatic control region,HCR)。在此基础上,通过分子克隆手段构建获得携带有不同数量的HCR增强子的嵌合型肝脏特异性hAAT启动子,并在下游连入报告基因Luciferase,然后将重组质粒转染人肝癌细胞系HepG2、小鼠肝癌细胞系Hepa1-6、人胚肾细胞系HEK293和人脑星形胶质母细胞瘤细胞系U87-MG,通过检测Luciferase表达活性分析携带不同数量增强子的肝脏特异性启动子的启动活性及其组织特异性。结果表明,携带有3个增强子的嵌合型肝脏特异性启动子活性及特异性最好,为肝脏类疾病的靶向性治疗研究奠定了基础。

关键词: 肝脏 增强子 特异性启动子 靶向治疗

The Study on Construction of Chimeric Hepatocyte Specific Promoter with Different Amount of Enhancers and Its Experimental Investigation *in Vitro*

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Abstract:

A specific promoter in hepatocytes--hAATp (human α1-antitrypsin promoter) and a HCR (hepatic control region) which can function as an enhancer using PCR were successfully obtained. On basis of this, several kinds of chimeric hepatocyte specific hAAT promoter with different amount of HCR enhancers were constructed. Then luciferase reporter gene was inserted into the downstream of the chimeric promoters. And the promoter activities of these different promoters and their specificity in hepatocytes were analyzed by detecting the expression activity of luciferase in different recombinant plasmid separately based on the transfection of the recombinant plasmids into HepG2 cells, Hepa1-6 cells, HEK293 cells and U87-MG cells. The result showed that among these reconstructed chimeric promoters, the one carrying three enhancer possess the best specificity in hepatocytes. These results lay the foundation for target gene therapy in human liver diseases.

Keywords: Liver Enhancer Specific promoter Targeting therapy

收稿日期 2011-08-15 **修回日期** 2011-10-13 **网络版发布日期**

分类号:

Q789

基金项目:

国家自然科学基金资助项目(30872993,31070137)

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