

论文

糖核酸内切酶III制备的小干扰RNA(esiRNA)文库对乙肝病毒感染的基因治疗

田明忠<sup>1</sup>, 高杰<sup>1</sup>, 王东秋<sup>1</sup>, 朱长军<sup>2</sup>

1.山东大学医学院细胞生物学研究所, 济南 250012;  
2.天津师范大学生命科学院, 天津 300387

摘要:

目的 建立HBV esiRNA文库并研究该文库对HepG2.215细胞内HBV表面抗原(HBsAg)表达的抑制效应。方法 制备并纯化大肠杆菌核糖核酸内切酶III GST 融合蛋白(GST RNaseIII);设计并制备HBV基因组序列1~540核苷酸(HBV-1)的双向转录模板(T7-HBV-1);体外转录该模板获得双链RNA(dsRNA),即T7-HBV-1 dsRNA;用GST-RNaseIII酶切T7-HBV-1 dsRNA制备HBV小干扰RNA文库(esiRNA library);应用50、100和150nmol/L纯化的esiRNA文库转染携带HBV病毒的HepG2.215细胞并应用实时定量PCR及ELISA检测HBsAg的转录和蛋白翻译表达水平。结果 成功制备并纯化有活性的GST-RNaseIII;用该酶将体外制备的dsRNA酶切并纯化得到esiRNA文库;应用该文库成功抑制了HepG2.215细胞内的HBV HBsAg的表达,并且其抑制作用随esiRNA浓度升高而增强。结论 用生物学方法制备的HBV esiRNA文库可以成功抑制HBV HBsAg在细胞内的表达,为预防治疗HBV感染开辟了新的思路。

关键词: RNA干扰;文库;肝炎病毒,乙型;核糖核酸内切酶III

Gene therapy of endoribonucleaseIII-prepared siRNAs (esiRNA) library for HBV infection

TIAN Ming-zhong<sup>1</sup>, GAO Jie<sup>1</sup>, WANG Dong-qiu<sup>1</sup>, ZHU Chang-jun<sup>2</sup>

1. Institute of Cell Biology, School of Medicine, Shandong University, Jinan 250012, China;  
2. School of Life Science, Tianjin Normal University, Tianjin 300387, China

Abstract:

Objective To construct a HBV esiRNA library and to study its inhibitory effects on HBV replication in HepG2.215 cells. Methods The GST-fusion protein of Escherichia coli endoribonucleaseIII (GST-RNase III) was prepared, purified, and then used to cleave the double strand RNA(dsRNA) transcribed in vitro from a two-orientation-transcription template for 1-540 nucleotides of the HBV genome(T7-HBV-1). The HBV esiRNA library generated from the above digestion was then used at 50, 100 and 150nmol/L to transfect HepG2.215 cells carrying HBV. The inhibitory effects on HBsAg expression were examined with Q RT and ELISA assays. Results Active enzyme GST-RNaseIII was prepared and purified. The HBV esiRNA library was successfully generated from dsRNA by GST RNaseIII digestion. HBsAg expression was significantly reduced in HepG2 cells after transfection of the esiRNA library. Conclusion Biologically prepared esiRNA library could successfully inhibit the replication of HBV in HepG2.215 cells, providing a novel idea for the treatment of HBV infection.

Keywords: RNA interference; Library; Hepatitis B virus; EndoribonucleaseIII

收稿日期 2010-01-06 修回日期 网络版发布日期

DOI:

基金项目:

国家自然科学基金青年科学基金项目(30700396);山东省科技攻关项目(2008GG10002019)

通讯作者:朱长军(1972-),男,教授、博士生导师,主要从事细胞周期研究。Email: zhucj1972@gmail.com

作者简介:田明忠(1982-),男,硕士研究生,主要从事细胞分子生物学研究。

作者Email:

参考文献:

扩展功能

本文信息

- ▶ Supporting info
- ▶ PDF(838KB)
- ▶ [HTML全文]
- ▶ 参考文献[PDF]
- ▶ 参考文献

服务与反馈

- ▶ 把本文推荐给朋友
- ▶ 加入我的书架
- ▶ 加入引用管理器
- ▶ 引用本文
- ▶ Email Alert
- ▶ 文章反馈
- ▶ 浏览反馈信息

本文关键词相关文章

- ▶ RNA干扰;文库;肝炎病毒,乙型;核糖核酸内切酶III

本文作者相关文章

PubMed

