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论著

利用纳米和RNA干扰技术抑制HBV-DNA在HepG2 2.2.15细胞中的复制和表达

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摘要：

目的：通过RNA干扰和纳米技术抑制HBV-DNA在体外的复制和HBV核心抗原的表达。方法：制备靶向HBV核心抗原（HBcAg）的纳米小干扰RNA（siRNA），利用U6启动子质粒转染入HepG2 2.2.15细胞；RT-PCR和Western印迹检测转染细胞HBV核心抗原在mRNA和蛋白水平的表达情况；real-time PCR 检测上清液HBV-DNA，放射免疫法检测细胞HBV表面抗原（HBsAg）、e抗原（HBeAg）、核心抗原（HBcAg）。结果：成功构建了含磁性纳米的siRNA质粒；多种方法检测均显示转染后的细胞HBV核心抗原表达明显下降；其表面抗原、e抗原和HBV-DNA值均较对照组降低。结论：RNA干扰联合纳米技术可明显下调HBV核心抗原的表达，抑制HBV-DNA复制。

关键词： 小干扰RNA HBV HBV核心抗原 磁性纳米

Inhibition of HBV-DNA replication and expression by siRNA based on magnetic nanoparticles transfering in HepG2 2.2.15 cells

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Abstract:

ObjectiveTo investigate the inhibitory effect of downregulation of hepatitis B virus (HBV) core gene (HBcAg） expression by RNA interference and magnetic nanoparticles on both HBV DNA replication and expression in vitro. MethodsHepG2 2.2.15 cells were transfected with U6 promoter plasmids coding for small interfering RNA (siRNA) targeting HBV core gene using magnetic nanoparticles. RT-PCR and Western blot were used to assess the mRNA and protein expression HBV core antigen. Real-time PCR was used to evaluate the suppression efficiency of HBV-DNA replication and expression; and radioimmunoassay was used for HBV surface antigen （HBsAg）, core antigen （HBcAg）, and e antigen （HBeAg） detection. ResultsWe successfully constructed nanoparticles with siRNA plasmid targeting HBV core antigen; HBcAg mRNA and HBV core antigen protein levels were significantly reduced in the transfected cells. HBV-DNA downregulation was estimated at 4-5 logs and the HBsAg and HBeAg levels were also reduced compared with the controls. ConclusionDownregulation of HBV core gene using RNAi technology and magnetic nanoparticles can potentially be used as a therapeutic strategy for Hepatitis B.

Keywords: siRNA;HBV;HBV core gene;magnetic nanoparticles

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