[1]谢艳,黄世峰,曹炬,等.HCV核心蛋白对HepG2细胞增殖的影响[J].第三军医大学学报,2013,35(06):482-486.

Xie Yan,Huang Shifeng,Cao Ju,et al.HCV core protein promotes cell proliferation in HepG2 cells[J].J Third Mil Med Univ,2013,35(06):482-486.

HCV核心蛋白对HepG2细胞增殖的影响(PDF)

《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 35 期数: 2013年第06期 页码: 482-486 栏目: 论著 出版日期: 2013-03-30

Title: HCV core protein promotes cell proliferation in HepG2 cells

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关键词: HCV核心蛋白; Wnt1; miR-152; HepG2细胞系

Keywords: HCV core protein; Wnt1; miR-152; HepG2 cells

分类号: R394-33; R73-354; R735.7

DOI: -

文献标识码: A

摘要: 目的 利用腺病毒表达系统，研究丙型肝炎病毒（hepatitis C virus，HCV）核心蛋白对HepG2细胞增殖、Wnt1以及microRNA152（miR-152）表达水平的影响。 方法 通过HEK293细胞扩增腺病毒获得高滴度的Ad-EGFP和Ad-HCV core，分别感染HepG2细胞后，通过RT-PCR和Western blot证实HCV核心蛋白在HepG2细胞中高效表达。MTT法、细胞周期测定和克隆形成实验检测HCV核心蛋白对HepG2细胞增殖的影响。SYBR探针荧光定量PCR和Western blot检测的HepG2-HCV core细胞中Wnt1和miR-152表达的变化。用miR-152 inhibitor转染HepG2细胞后，SYBR探针荧光定量PCR、Western blot检测HepG2细胞中Wnt1 mRNA和蛋白表达水平变化。 结果 Ad-GFP和Ad-HCV core腺病毒经HEK293扩增后滴度分别为1.5×10^{9} pfu/mL和1.6×10^{10} pfu/mL。Ad-HCV core腺病毒感染HepG2细胞后，HCV核心蛋白高效表达。感染48 h后，与HepG2-Mock细胞相比，Ad-HCV core可显著促进HepG2细胞增殖（$P<0.05$），加快G_1/S细胞周期进展（$P<0.05$），并促进细胞克隆形成（$P<0.05$）。Ad-HCV core腺病毒感染可在显著上调HepG2细胞中Wnt1 mRNA和蛋白表达量（$P<0.05$）的同时下调miR-152表达水平。同时，miR-152 inhibitor可显著上调Wnt1 mRNA和蛋白水平。进一步生物信息学分析显示，Wnt1基因mRNA 3′-UTR含有miR-152结合位点。 结论 HCV核心蛋白可能通过抑制miR-152而减弱其对Wnt1 mRNA的降解及翻译阻碍作用，进而达到上调Wnt1致Wnt信号通路激活，促进肝癌细胞增殖的效应。

Abstract: Objective To determine the effect of HCV core protein on HepG2 cells proliferation after construction of a retroviral vector containing the protein, and detect the expression of Wnt1 and microRNA152 in the HepG2 cells after transfection. Methods A recombinant adenovirus expressing HCV core

protein along with EGFP with high titer was used to transfect the HepG2 cells. The expression of HCV core protein in the transfected cells was evaluated by real-time PCR and Western blot analysis. Cell viability of HepG2 cells was measured by MTT assay, flow cytometry and colony formation assay after the transfection of Ad-EGFP and Ad-HCV core. Real-time PCR and Western blot analysis was employed to detect the expression of Wnt1 and miR-152 in HepG2 cells transfected with HCV core protein and miR-152 inhibitor. Results The titer of the recombinant adenovirus expressing HCV core was 1.6×10^{10} pfu/mL, and that of Ad-EGFP was 1.5×10^{9} pfu/mL The recombinant viruses could effectively transfected into HepG2 cells, and HCV core protein was highly expressed after the transfection of Ad-HCV core. The transfection of the virus Ad-HCV core resulted in an increase in cell proliferation, and promotion in G_1/S cell cycle progression and formation of cell colonies in 48 h after transfection (P<0.05). Transfection of the adenovirus of HCV core caused up-regulation of Wnt1 and down-regulation of miR-152 (P<0.05). miR-152 inhibitor also upregulated the expression of Wnt1 at mRNA and protein levels (P<0.05). Subsequent bioinformative analysis revealed that the 3′-UTR of Wnt1 mRNA contained a complementary site for miR-152. Conclusion HCV core protein might attenuate the degradation and transcription of Wnt1 mRNA through inhibiting miR-152, and then activate the Wnt signaling pathway in order to promote the proliferation of hepatoma carcinoma cells.

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备注/Memo: -