

## 丙型肝炎病毒对高尔基体蛋白73表达的影响

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**【摘要】目的** 探讨丙型肝炎病毒(HCV)对高尔基体蛋白73(GP73)表达的影响。**方法** 选取2015年3月至2016年12月期间上海市浦东新区公利医院收治的68例HCV患者为研究对象,以同期74例健康体检者为对照组,采用酶联免疫吸附试验(ELISA)检测两组受检者血清中GP73的含量,逆转录-聚合酶链式反应(RT-PCR)和免疫印迹法检测Huh7.5.1细胞中GP73 mRNA和蛋白的表达情况,ELISA检测细胞上清中GP73的含量,用SPSS19.0软件进行统计学分析。**结果** HCV组患者的GP73血清含量为( $146.5 \pm 12.8$ ) ng/mL,明显高于健康对照组的( $45.2 \pm 2.7$ ) ng/mL,差异有统计学意义( $P < 0.05$ );感染HCV的Huh7.5.1细胞中GP73 mRNA和蛋白相对表达量分别为( $1.204 \pm 0.143$ )和( $1.174 \pm 0.136$ ),明显高于对照细胞的( $0.306 \pm 0.019$ )和( $0.298 \pm 0.015$ ),差异均具有统计学意义( $P < 0.05$ );HCV感染的细胞上清中GP73含量为( $85.6 \pm 10.3$ ) ng/mL,明显高于对照细胞的( $39.5 \pm 8.3$ ) ng/mL,差异具有统计学意义( $P < 0.05$ )。**结论** HCV可能通过上调GP73的表达来参与HCC的形成,但HCV调节GP73表达的具体分子机制尚待进一步研究。

**【关键词】** 丙型肝炎病毒;高尔基体蛋白73;表达

**【中图分类号】** R512.6<sup>+</sup><sup>3</sup>   **【文献标识码】** A   **【文章编号】** 1003—6350(2018)05—0657—03

**Effect of hepatitis C virus on the expression of Golgi protein 73.** LIU Xing-hui<sup>1</sup>, XU Feng-xia<sup>1</sup>, CHEN Sha-li<sup>1</sup>, SONG Hui<sup>1</sup>, LI Long-xuan<sup>2</sup>, LIU Fang<sup>3</sup>. 1. Department of Clinical Laboratory, Gongli Hospital of Pudong New Area of Shanghai, Shanghai 200135, CHINA; 2. Department of Neurology, Gongli Hospital of Pudong New Area of Shanghai, Shanghai 200135, CHINA; 3. College of Life Sciences, Wuhan University, Wuhan 430060, Hubei, CHINA

**[Abstract]** **Objective** To explore the effect of hepatitis C virus (HCV) on the expression of Golgi protein 73 (GP73). **Methods** A total of 68 HCV patients who were diagnosed in Gongli Hospital of Pudong New Area of shanghai from March 2015 to December 2016 were selected as research objects, and 74 healthy persons were enrolled as control group in the same period. Serum level of GP73 was measured by enzyme-linked immunosorbent assay (ELISA) between the two groups. Reverse transcription PCR (RT-PCR) and Western blot were used to measure mRNA and protein expression of GP73 in Huh7.5.1 cells. The GP73 level in the supernatant of Huh7.5.1 was measured by ELISA, and the results were statistically analyzed by SPSS19.0 software. **Results** The serum level of GP73 was ( $146.5 \pm 12.8$ ) ng/mL in HCV group, which was significantly higher than ( $45.2 \pm 2.7$ ) ng/mL in the healthy control group ( $P < 0.05$ ); The relative expression of GP73 mRNA and protein in Huh7.5.1 cells infected by HCV were respectively ( $1.204 \pm 0.143$ ) and ( $1.174 \pm 0.136$ ), which were significantly higher than ( $0.306 \pm 0.019$ ) and ( $0.298 \pm 0.015$ ) in the control cells ( $P < 0.05$ ); The GP73 level in the supernatant of Huh7.5.1 cells infected by HCV was ( $85.6 \pm 10.3$ ) ng/mL, which was significantly higher than ( $39.5 \pm 8.3$ ) ng/mL in the control cells ( $P < 0.05$ ). **Conclusion** HCV can promote the synthesis and secretion of GP73 in vitro and in vivo. It may participate in the formation of hepatocellular carcinoma by upregulating the expression of GP73, but the molecular mechanism of HCV regulating GP73 expression needs further study.

**[Key words]** Hepatitis C virus (HCV); Golgi protein 73 (GP73); Expression

丙型肝炎病毒(hepatitis C virus,HCV)感染机体后引起肝脏的急慢性炎症,发展为肝硬化,最终导致原发性肝细胞癌(hepatocellular carcinoma,HCC)的发生。迄今为止,全球大约已有180万的HCV感染者,相当一部分人发展成为慢性HCV感染,其中1%~5%的慢性HCV患者最终发展成为HCC<sup>[1]</sup>。高尔基体蛋白73(Golgi protein 73,GP73)是一种新的用于诊断肝脏疾病和HCC的血清标志物。前期研究表明,乙型肝炎病毒(hepatitis B virus,HBV)能够促进GP73的表达,并且GP73的血清含量与HBV相关疾病的进程存在一定的正相关<sup>[2]</sup>。本研究拟通过体内外实验探讨HCV对GP73表达的调节作用,旨在为揭示HCV致病及致癌

机理提供理论基础。

### 1 资料与方法

1.1 一般资料 收集2015年3月至2016年12月期间上海市浦东新区公利医院临床确诊的门诊和住院HCV感染患者68例,其中男性42例,女性26例,平均年龄( $43.5 \pm 11.7$ )岁。收集同期74例健康体检者作为对照组,其中男性46例,女性28例,平均年龄( $44.2 \pm 13.3$ )岁。所有患者均排除HBV、戊型肝炎病毒、流感病毒、支原体和衣原体等其他病原体感染。

1.2 材料 含JFH1亚型的HCV感染的Huh7.5.1细胞由本室保存<sup>[3]</sup>;GP73 ELISA检测试剂盒和TRIzol R试剂均购自Invitrogen公司;M-MLV逆转录酶购自

基金项目:上海市卫生和计划生育委员会科研课题项目(编号:201440459);上海市医学重点专科建设计划项目(编号:ZK2015B16);浦东新区卫 生系统重点学科群建设资助项目(编号:PWZxq2017-15)  
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### 1.3 方法

1.3.1 细胞培养 Huh7.5.1细胞培养在含10%的胎牛血清(foetal bovine serum, FBS)、100 U/mL 青霉素和100 mg/L链霉素的DMEM培养基中,在CO<sub>2</sub>浓度为5%、温度为37℃的细胞培养箱中进行培养。

1.3.2 RT-PCR检测 收集HCV感染的Huh7.5.1细胞及其对照细胞,加入1 mL TRIzol R试剂,提取细胞的总RNA,逆转录酶M-MLV将mRNA逆转录生成DNA后,用GP73基因的引物进行PCR扩增,上游引物:5'CGTCTTGGGCTCAACTAC3',下游引物:5'TCTTCTGAAACTGGAGGACAT3',并设立扩增β-actin作为参照,PCR扩增产物用2%琼脂糖凝胶进行电泳检测。

1.3.3 免疫印迹法检测 Huh7.5.1细胞经超声破碎后取细胞上清,加入等体积上样缓冲液煮沸,用12%十二烷基硫酸钠聚丙烯酰胺凝胶电泳进行电泳分离,电泳结束后将蛋白转至硝酸纤维素膜上,分别加入浓度为1:2 000的GP73蛋白多克隆抗体和1:5 000的羊抗兔二抗,采用电化学发光法显色。

1.3.4 EELISA检测 采用ELISA法检测血清和细胞上清中GP73的含量,按照GP73 ELISA试剂盒说明书的操作方法进行,实验重复3次。

1.4 统计学方法 应用SPSS19.0统计学软件进行统计学分析,计量资料以均数±标准差( $\bar{x}\pm s$ )表示,两组间数据比较采用t检验,以P<0.05为差异有统计学意义。

## 2 结果

2.1 HCV患者GP73血清含量升高 采用ELISA检测了HCV患者和健康对照组的GP73血清含量,结果表明,GP73在HCV组为(146.5±12.8) ng/mL,明显高于健康对照组的(45.2±2.7) ng/mL,差异有统计学意义(P<0.05)。

2.2 HCV在Huh7.5.1细胞促进GP73的合成和分泌 采用RT-PCR和免疫印迹法检测了含HCV JFH1亚型的Huh7.5.1细胞和未感染HCV的Huh7.5.1细胞中GP73 mRNA和蛋白的表达水平。结果发现,含HCV JFH1亚型的Huh7.5.1细胞中GP73 mRNA和蛋白的相对表达量分别为(1.204±0.143)和(1.174±0.136),明显高于对照组的(0.306±0.019)和(0.298±0.015),差异有统计学意义(P<0.05),见图1和图2。进一步EELISA检测结果显示,HCV感染的Huh7.5.1细胞上清中GP73含量为(85.6±10.3) ng/mL,明显高于对照细胞的(39.5±8.3) ng/mL,差异有统计学意义(P<0.05)。

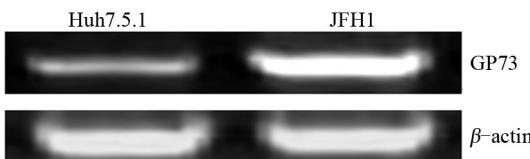


图1 RT-PCR检测GP73 mRNA的表达

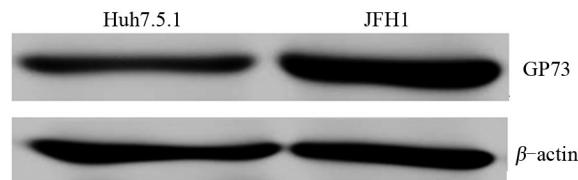


图2 免疫印迹法检测GP73蛋白的表达

### 3 讨论

GP73是分子量大小为73 kD的高尔基体膜蛋白,由401个氨基酸组成,在病毒性肝炎和非病毒性肝炎患者血清和肝组织中高表达。GP73作为一种新的肝癌诊断标志物,与传统的甲胎蛋白(α-fetoprotein, AFP)和白细胞介素6(interleukin 6, IL-6)等指标相比,具有更高的灵敏度、特异性和准确性<sup>[4]</sup>。

研究表明,很多病毒感染能够调节GP73的表达如HBV、巨细胞病毒(cytomegalovirus, CMV)和人类免疫缺陷病毒(human immunodeficiency virus, HIV)等。此外,GP73在HCC患者的肝癌组织中表达也升高<sup>[5-7]</sup>。目前关于HCV与GP73的相关研究较少。本研究主要在临床和细胞水平探讨了HCV对GP73表达的调节作用。

研究结果显示,HCV感染患者血清GP73的表达水平升高,表明HCV能够在体外上调GP73的表达。HCV感染与复制最常用的细胞模型是Huh7.5.1,本研究进一步分析了JFH1亚型的HCV感染的Huh7.5.1细胞及其对照细胞中GP73的表达差异,并检测了细胞上清中GP73的含量。结果表明,HCV能够在细胞水平促进GP73的合成和分泌。目前,大量研究主要集中于GP73对HCV复制影响的方面。Zhang等<sup>[8]</sup>研究证明GP73能够和HCV非结构蛋白5A(nonstructure 5A, NS5A)及载脂蛋白E(apolipoprotein E, ApoE)相互作用,促进HCV的复制。

HCV是诱发HCC的主要病原体之一,但目前为止,HCV引发肝癌的机制尚未完全明确,GP73在肿瘤的发生和发展过程中起到重要作用。裸鼠成瘤实验表明,GP73具有促进体外肝细胞癌增殖、分化和转移的功能,可以通过激活人基质金属蛋白酶-13(matrix metalloproteinase, MMP-13)来促进肝癌细胞的侵袭<sup>[9]</sup>,干扰掉GP73的表达后能够促进肝细胞癌的凋亡从而抑制其增殖<sup>[10]</sup>。本研究证实了HCV能够在体内外促进GP73的合成和分泌。因此,HCV可能通过上调GP73的表达来参与HCC的形成,但HCV调节GP73表达的具体分子机制尚待进一步研究。

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## 2型糖尿病患者血脂与尿微量蛋白肌酐比的相关性研究

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**【摘要】目的** 探讨2型糖尿病患者血脂与尿微量蛋白肌酐比(UACR)的相关性。**方法** 回顾性分析2014年3月至2016年3月秦皇岛市第一医院70例2型糖尿病患者的临床资料,设为糖尿病组,另选取同期健康体检者70例为对照组。比较两组受检者的甘油三酯(TG)、总胆固醇(TG)、高密度脂蛋白(HDL-C)、低密度脂蛋白(LDL-C)等血脂指标以及尿微量蛋白(UALB)、尿肌酐(CR)、血尿素氮(BUN)、UACR等肾功能指标,采用Spearman相关分析探讨血脂指标与肾功能指标之间的关系。**结果** 糖尿病组患者的TC、TG和LDL-C水平分别为( $5.81\pm1.36$ ) mmol/L、( $2.53\pm0.72$ ) mmol/L、( $3.52\pm0.85$ ) mmol/L,均明显高于对照组的( $4.32\pm1.12$ ) mmol/L、( $1.24\pm0.19$ ) mmol/L、( $2.85\pm0.16$ ) mmol/L, HDL-C水平为( $1.15\pm0.52$ ) mmol/L,明显低于对照组的( $1.47\pm0.22$ ) mmol/L,差异均有统计学意义( $P<0.05$ );糖尿病组患者的UALB、CR、BUN、UACR水平分别为( $24.17\pm12.20$ ) mg/L、( $83.34\pm26.34$ )  $\mu$ mol/L、( $7.12\pm2.56$ ) mmol/L、( $13.46\pm5.69$ ),均明显高于对照组的( $16.32\pm3.27$ ) mg/L、( $72.36\pm16.35$ )  $\mu$ mol/L、( $4.37\pm2.10$ ) mmol/L、( $5.61\pm1.65$ ),差异均有统计学意义( $P<0.05$ );TC、TG水平与UACR呈正相关( $r=0.079, 0.062, P<0.05$ ),HDL-C、LDL-C水平与UACR无显著相关性( $r=-0.085, 0.087, P>0.05$ )。**结论** 2型糖尿病患者伴有一定血脂紊乱及肾功能损伤,且血脂水平中TC、TG与UACR存在相关性。

**【关键词】** 2型糖尿病;血脂;尿微量蛋白肌酐比;相关性

**【中图分类号】** R587.1   **【文献标识码】** A   **【文章编号】** 1003—6350(2018)05—0659—03

**Correlation between blood lipid and urine albumin-to-creatinine ratio in patients with type 2 diabetes mellitus.**  
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**[Abstract]** **Objective** To investigate the correlation between blood lipid and urine albumin-to-creatinine ratio (UACR) in patients with type 2 diabetes mellitus. **Methods** The clinical data of 70 patients with type 2 diabetes mellitus in the First Hospital of Qinhuangdao City from March 2014 to March 2016 were analyzed retrospectively (diabetic group). At the same time, 70 healthy individuals were selected as control group. The indexes of blood lipids such as triglyceride (TG), total cholesterol (TG), high-density lipoprotein (HDL-C), low density lipoprotein (LDL-C), and renal function indexes such as urinary microprotein (UALB), creatinine (CR), blood urea nitrogen (BUN) and UACR were compared between the two groups. Spearman correlation analysis was used to investigate the relationship between blood lipid indexes and renal function indexes. **Results** TC, TG and LDL-C levels were ( $5.81\pm1.36$ ) mmol/L, ( $2.53\pm0.72$ ) mmol/L, ( $3.52\pm0.85$ ) mmol/L in the diabetic group, which were significantly higher than ( $4.32\pm1.12$ ) mmol/L, ( $1.24\pm0.19$ ) mmol/L, ( $2.85\pm0.16$ ) mmol/L in the control group, while HDL-C level was ( $1.15\pm0.52$ ) mmol/L in the diabetic group, significantly lower than ( $1.47\pm0.22$ ) mmol/L in the control group ( $P<0.05$ ). UALB, CR, BUN, UACR levels were ( $24.17\pm12.20$ ) mg/L, ( $83.34\pm26.34$ )  $\mu$ mol/L, ( $7.12\pm2.56$ ) mmol/L, ( $13.46\pm5.69$ ) in the diabetic group, significantly higher than ( $16.32\pm3.27$ ) mg/L, ( $72.36\pm16.35$ )  $\mu$ mol/L, ( $4.37\pm2.10$ ) mmol/L, ( $5.61\pm1.65$ ) in the control group ( $P<0.05$ ). TC and TG were positively correlated with UACR ( $r=0.079, 0.062, P<0.05$ ), and there was no significant correlation between HDL-C, LDL-C and UACR ( $r=-0.085, 0.087, P>0.05$ ). **Conclusion** Type 2 diabetes mellitus is associated with certain dyslipidemia and renal dysfunction, and there is a correlation between TC, TG and UACR.

**【Key words】** Type 2 diabetes mellitus; Blood lipid; Urine albumin-to-creatinine ratio; Relevance

基金项目:河北省秦皇岛市科技支撑项目(编号:201502A168)

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(收稿日期:2017-06-27)