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论著

筛选丙型肝炎病毒1b型非结构蛋白4B慢病毒L02稳定株差异表达基因和基因通路

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摘要：

目的：筛选表达丙型肝炎病毒(hepatitis C virus，HCV)1b型非结构蛋白4B(nonstructural protein 4B，NS4B)的慢
病毒稳定细胞株L02-NS4B差异表达基因和基因通路，为深入研究HCV NS4B在慢性丙型肝炎和肝细胞癌发生中的作用
及机制提供依据。方法：将已构建好的2株慢病毒稳定细胞株LO2-NS4B和阴性对照慢病毒稳定细胞株LO2-mkate2复苏
扩增；应用Human Gene 1.0ST 芯片筛选出LO2-NS4B与LO2-mkate2差异表达基因。基于京都基因和基因组百科全书(kyoto
encyclopedia of genes and genomes，KEGG)数据库，利用Fisher精确检验和卡方检验，对差异基因参与的信号转导通路进行
显著性分析。应用实时定量聚合酶链反应(real-time quantitative polymerase chain reaction，real-time QPCR)方法验证基因芯片
中5个表达上调且与凋亡有关的基因，即蛋白激酶C 结合蛋白(protein kinase C delta binding protein，PRKCDBP)基因、肿瘤
蛋白p53(tumor protein p53，TP53)基因、v-akt 鼠科胸腺瘤病毒癌基因同源物1 (v-akt murine thymoma viral oncogene homolog 1，AKT1)基因、含3个杆状病毒凋亡蛋白抑制因子重复序列 (baculoviral IAP repeat containing 3，BIRC3)基因和B细胞淋巴瘤2样1基因(B-cell lymphoma 2-like1，BCL2L1)的mRNA水平。结果：以LO2-NS4B与LO2-mkate2之间基因荧光强度的比值大于1.2或小于0.8为差异表达基因，在已知的28 869个人类基因中L02-NS4B中有2 682个差异表达基因，包括1 446个基因表达上
调和1 236个基因表达下调。上调基因参与的显著性信号转导通路41项，主要有凋亡通路、细胞外基质受体相互作用
通路、细胞周期通路、癌症通路和Toll样受体信号通路等；下调基因参与的显著性信号转导通路20项，主要有癌症通
路、Wnt信号通路和细胞周期通路等。Real-time QPCR验证5个表达上调基因中有3个基因表达变化与基因芯片结果一
致，分别是AKT1，BIRC3和BCL2L1，吻合率为60%。结论：HCV NS4B可以调节LO2细胞中与细胞凋亡、细胞周期和
细胞增殖等有关的多种基因表达，主要影响与细胞凋亡、细胞周期和癌症相关的信号转导通路。

关键词： 丙型肝炎病毒 非结构蛋白4B 基因芯片 慢病毒

Screening of differentially expressed genes and gene pathways in hepatitis C virus 1b type nonstructural protein 4B stably expressed L02 cell line

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Abstract:

Objective: To screen differentially expressed genes and gene pathways in L02 cell line stably expressing hepatitis C virus (HCV) Ib type nonstructural protein 4B (NS4B) mediated by lentiviral system, and to provide a basis for further research of molecular biological mechanism of NS4B gene in chronic hepatitis C and hepatocarcinogenesis.
Methods: NS4B stably overexpressed L02 cell line and negative control stable L02 cell line, designated as L02-NS4B and L02-mkate2 respectively, were resurrected and amplified in vitro. The differentially expressed genes between L02-NS4B and L02-mkate2 were determined by gene expression microarray from Human Gene 1.0ST. The significant pathways of the differential genes were selected by the Fisher’s exact test and χ2 test according to kyoto encyclopedia of genes

and genomes (KEGG) database. The differential expression levels of 5 selected genes including protein kinase C delta binding protein (PRKCDBP), tumor protein p53 (TP53), v-akt murine thymoma viral oncogene homolog 1 (AKT1), baculoviral IAP repeat containing 3 (BIRC3) and B-cell lymphoma 2-like1 (BCL2L1) from cDNA microarray data were further verified by real-time quantitative polymerase chain reaction (real-time QPCR).
Results: Between L02-NS4B and L02-mkate2, the genes with flurescence intensity ratio >1.2 or <0.8 were considered as differentially expressed genes. A total of 2 682 differentially expressed genes in the known 28 869 human genes were detected in L02-NS4B, 1 446 genes were upregulated and 1 236 genes were downregulated. A total of 41 involved pathways of up-regulated differential genes were identified by KEGG database, mainly including apoptosis, extracellular matrix receptor interaction, cell cycle, pathways in cancer and Toll-like receptor signaling pathway; and 20 involved pathways of down-regulated differential genes were identified, mainly including pathways in cancer, Wnt signaling pathway and cell cycle pathway. Of the 5 upregulated genes selected from cDNA microarray data, 3 genes showed the same differential expression pattern by real-time QPCR as that shown in cDNA microarray data, namely AKT1, BIRC3 and BCL2L1. The confirmation rate of real-time QPCR was 60%.
Conclusion: HCVNS4B can up-regulate or down-regulate the expression of many genes in L02 cells, thus affecting multiple signaling pathways relevant to cell apoptosis, cell cycle and carcinogenesis.

Keywords: hepatitis C virus nonstructural protein 4B microarray lentivirus

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