Original Article
Mechanism of ribavirin in treatment of hepatocellular carcinoma via microarray analysis

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Abstract: In order to explore the molecular mechanism of ribavirin affected hepatocellular carcinoma (HCC) via microarray analysis. Microarray data set GSE23031 was downloaded from Gene Expression Omnibus (GEO), which contained 6 samples, 3 cultured cells treated with ribavirin as case groups and 3 cultured cells treated with phosphate buffer solution (PBS) as control groups. Differentially expressed genes (DEGs) were screened out by the Affy package of R. Gene Ontology (GO) enrichment analysis and signal pathway analysis were performed via the Database for Annotation, Visualization and Integrated Discovery (DAVID). Protein-protein interaction (PPI) network and modules were constructed by Protein Interaction Network Analysis (PINA) and CMODE plug-in of Cytoscape software, respectively. A total of 126 DEGs (43 up- and 83 down-regulated) were obtained. Response to organic substance and response to hormone stimulus were the significantly enriched GO terms. PPI network indicated that CDC45, CCND1 and HDAC9 were related to hepatocellular carcinoma. Two modules and some signal pathways were obtained, it demonstrated that ribavirin played its role mainly via small cell lung cancer signal pathway and metabolism of lipids and lipoproteins signal pathway. Our study provided new data to understand the mechanism of ribavirin in vivo. It could be valuable for the subsequent study.

Keywords: Ribavirin, hepatocellular carcinoma, differentially expressed genes, protein-protein interaction (ppi) network

Introduction

Hepatocellular carcinoma (HCC) is a common cancer originating from liver cells. Every year, the incidence of HCC is increasing and accounting for approximately 800 thousand deaths [1]. It is currently the second leading cause of cancer-related death worldwide [2]. Viral infection is mostly the chief cause of HCC. Hepatitis C viruses (HCV) usually escape the host antiviral response and develop chronic hepatitis, which can lead to progressive liver disease such as cirrhosis, liver failure and HCC [3]. While chronic hepatitis B virus (HBV) infection is a critical risk factor for the carcinogenesis and progression of HCC in China [4].

Ribavirin is a guanosine analog that shows an extensive antiviral activity in tissue culture. It is widely used in the treatment for chronic hepatitis virus infection at present. Previous research reported ribavirin successfully used in the management of chronic infection of hepatitis E virus (HEV) [5]. The combination of pegylated interferon (PEG-IFN) and ribavirin therapy is the current therapy for chronic hepatitis C. Trials of dual therapy with interferon (IFN) and ribavirin displayed more effectively than monotherapy [6]. When ribavirin was used as monotherapy, it had transient antiviral activity in patients [7], but when combined with IFN, it markedly improved treatment response [8]. Although it is well-known that ribavirin could improve treatment outcomes, the precise mechanism how it affected HCC has remained elusive. Several mechanisms of ribavirin’s antiviral activity have been proposed, but none has been convinced [9]. Lately, ribavirin has been shown to modulate the expression of interferon-stimulated
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The Database for Annotation, Visualization and Integrated Discovery (DAVID) [11] was used for GO enrichment analysis of DEGs. The GOTERM_BP_FAT, GOTERM_MF_FAT and GOTERM_CC_FAT that means biological processes (BP), molecular functions (MF) and cellular component (CC) respectively and they were chosen to investigate functional classification. The P value less than 0.05 was set as cut-off criterion.

PPI network construction and visualization

Protein Interaction Network Analysis (PINA) platform is an integrated platform for protein interaction network construction, filtration, analysis, visualization and management. With integrating six public databases, it built a complete, non-redundant PPI dataset for six model organisms. Moreover, it provided a variety of built-in tools to filter and analyze the network for gaining insight into the network. What we need are the Homo sapiens data which contain 15663 proteins and 108477 interactions. PINA2 [12] (http://cbg.garvan.unsw.edu.au/pina/home.do) database was chosen to construct PPI network. Cytoscape is an open source software platform for visualizing biological pathways and molecular interaction networks and integrating these networks with annotations, gene expression profiles and other state data. Now it is a general platform for complex network analysis and visualization [13]. Here, the PPI network was visualized through Cytoscape.

Module analysis

Models are developed to simulate biochemical reactions and gene transcription kinetics, cellular physiology and metabolic control. Modules provide a framework for managing immense complexity of cellular components and interactions and revealing nascent properties and unsuspected consequences of different pathway configurations [14]. In this study, module

<table>
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<th>P value</th>
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<td>Response to hormone stimulus</td>
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<td>Response to insulin stimulus</td>
<td>4</td>
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<td>Mitotic cell cycle checkpoint</td>
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GO, Gene Ontology.
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Pathway construction

Pathway analysis makes it possible to reveal the molecular interaction and reaction networks for metabolism, genetic information processing, environmental information processing, cellular processes, organism systems and human diseases. In order to investigate the integrate mechanism of ribavirin affected HCC, relevant pathways were obtained by DAVID.

Results

Differentially expressed genes

After the treatment of ribavirin, a total of 126 DEGs were screened out which contained 43 up-regulated genes and 83 down-regulated genes in cultured cells (treated with ribavirin) compared with control groups.

GO enrichment analysis of DEGs

GO enrichment analysis could provide functional annotation and classification for analyze the
gene sets data. The screened DEGs were enriched in 48 GO terms, including 35 BP, 9 MF and 3 CC according to the functional annotation. The top 10 significantly GO terms were listed in Table 1.

Table 1 showed that GO terms for biological processes significantly enriched in response to organic substance (GO: 0010033) and response to hormone stimulus (GO: 0009725). Meanwhile, cyclin D1 (CCND1) and histone deacetylase 9 (HDAC9) had been found frequently enriched in many GO terms.

PPI network construction

Based on the PINA2 database, a total of 983 genes and 1173 interactions were identified in the PPI network. Visualized PPI network was shown in Figure 1. In this network, 62 DEGs were marked yellow and 921 non-differential express genes were marked blue. Degree was used to describe the numbers of one gene directly interact with other genes [15]. The top 10 genes with the largest degrees were listed in Table 2. In Table 2, CDC45, CCND1, HDAC9 and CHAF1A had higher scores and CDC45 had the highest degree while it was not a DEG.

Module analysis

The CMODE plug-in of Cytoscape was used to perform the module analysis. Two significant modules were obtained when the score cut-off value was no less than 2 (Table 3). In module 1, Prostate tumor overexpressed 1 (PTOV1) had direct correlation with the other DEGs, and INF2 was the core gene in module 2.

Pathway construction

In order to further understand the effect of ribavirin in the therapy of HCC, we constructed the pathways by DAVID. One KEGG pathway (small cell lung cancer) and one REACTOME pathway (metabolism of lipids and lipoproteins) were obtained and they were listed in Table 4.

Discussion

Many causes of HCC were related to virus infection such as HBV, HCV and HEV, therefore, the antiviral treatments has become critical in the HCC therapy. Ribavirin as an antiviral agent has been widely used in clinical liver diseases treatment and shown effectively outcome. In this paper, we reanalyzed the available GEO microarray data to research the mechanism of ribavirin in HCC treatment. Our results indicated that identified DEGs either in biological roles or in signal pathways analysis showed high correlation between hepatic disease and tumors. The significantly GO terms, such as response to organic substance and response to hormone stimulus, were both related to liver diseases. Gotohda T et al. [16] studied toluene inhalation primarily induced hepatic damage. Moos RH et al. [17] described remission of substance abuse delayed mortality of liver cirrhosis. Psarra AM et al. [18] stated that one main site of action of glucocorticoids was the hepatocyte, which responded to the hormonal stimulus. Nov O et al. [19] suggested that adipocyte-derived IL-1 beta might constitute a mediator in the perturbed cross talk between adipocytes and liver cells in response to adipose tissue inflammation. Collis SJ et al. [20] focused on how biological clock-associated proteins were involved in, through their interplay with cell cycle/DNA damage response pathways, in the development of human disease such as cancer. These above views could be the evidences that the DEGs enriched in GO terms were mostly related to liver metabolic processes. Therefore, we believed that ribavirin might play its function via these processes.
In our study, the DEGs with highest degree score, such as CDC45, CCND1, HDAC9 and CHAF1A, might be associated with human cancer. Although CDC45 is a non-differential expressed gene, the highest degree implied it had a potential role in oncogenesis. CDC45 is considered as the essential DNA replication factor to ensure that chromosomal DNA can be replicated regularly during cell cycle. S. Pollok et al. [21] demonstrated that CDC45 protein level was positively correlated with human cancer-derived cells and suggested it could be a biomarker to help assess tumor proliferation and the potential prognosis of tumorigenic diseases. CCND1 was also involved in cell cycle regulation and DNA synthesis. It has been shown to be related to mammary gland epithelial cell proliferation, liver development, cell division and response to estrogen stimulus, which could lead to breast cancer and liver cancer [22]. Crosas-Moolist E et al. [23] and Liu Y et al. [24] researched the relationship between CCND1 and liver cancer. The results shown that CCND1 played a role in metabolism linked to proliferation and cancer. HDAC9 is a member of the histone deacetylase family, and is known to play an important role in carcinogenesis due to epigenetic modification of DNA or the histone proteins [25]. Dong YW et al. [26] and Archer KJ et al. [27] had already studied the relationship between HDAC9 and HCC.

Two modules were successfully constructed, and PTOV1 enriched in module 1 which had proved it was connected with multiple cancers including lung, endometrium, bladder, kidney and ovary cancer [28]. Followed by signal pathway analysis, we uncovered the underlying mechanisms of ribavirin in the progression of HCC. In our study, the DEGs were enriched in small cell lung cancer signal pathway and metabolism of lipids and lipoproteins signal pathway. Small cell lung carcinoma (SCLC) is a highly aggressive neoplasm. MYC was known to be induced expression of oncogene in SCLC, and the overexpression of MYC proteins was largely a result of gene amplification, while loss of tumor suppressor genes, such as p53, PTEN, RB and FHIT also could lead to tumorigenesis [29]. Kim HJ et al. [30] and Stitziel NO et al. [31] had reported the correlation of metabolism of lipids and lipoproteins with HCC. According to many evidence, we speculated these two pathways were reliable, and ribavirin might augment interferon-stimulated gene induction to a great extent via these two pathways.

In conclusion, by microarray analysis, we have identified DEGs, GO terms, modules and signal pathways in ribavirin treatment groups compared with control groups, which could provide understanding in the effect of ribavirin in HCC. These genes (e.g. CDC45, CCND1 and HDAC9) might be the key ones in the progress of ribavirin affecting HCC, and they will contribute to the treatment of HCC. However, further study is still needed to confirm the functions of these key genes in HCC.

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Disclosure of conflict of interest

None.

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