Exploration and review of the molecular mechanisms of hepatitis C virus infection-induced hepatocellular carcinoma

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Abstract. Hepatitis C virus (HCV) is a virus that causes acute and chronic hepatitis, which can progress to liver damage. The link between HCV infection and hepatocellular carcinoma (HCC) has been proven by many studies. Long-term inflammation caused by HCV infection is one of the carcinogenic factors. Secondly, HCV infection significantly changes the expression of many specific genes and signaling pathways in hepatocytes. The signaling pathways affected by HCV infection have been found to be related to cellular defense mechanisms (apoptosis, proliferation, and antioxidant responses), cellular metabolism (lipid and protein metabolism), and intracellular transport (vesicles). Changes caused by HCV tend to persist and are associated with liver carcinogenesis even after cure, as evidenced by the subsequent development of HCC that persists after clearance of HCV. This study employed transcriptome sequencing data from public databases for gene enrichment analysis. The results were subsequently compared with the findings in the literature review. As a result, it was observed that HCV infection increases the risk of hepatocellular carcinoma by altering the gene expression associated with the PI3K/AKT/mTOR pathway, cellular apoptosis, protein synthesis, and intracellular transport.

1 Introduction

Hepatitis C virus (HCV) is an RNA virus with liver specificity that can cause both acute and chronic hepatitis, resulting in progressive liver damage. HCV infections initially manifest as acute, often with mild symptoms, but approximately 80% of patients ultimately develop persistent chronic infections, leading to steatosis, fibrosis, cirrhosis, liver failure, and, in some cases, hepatocellular carcinoma (HCC) [1]. In recent advances in antiviral drug therapy, sustained virological response rates of over 95% for HCV have been achieved [2]. However, there is also evidence indicating that the clearance of the hepatitis C virus may not prevent the subsequent development of HCC [3]. Research on how HCV infection contributes to the risk of HCC remains inconclusive, but data from related studies suggest that the incidence of HCC is 5-10% 20 years after HCV infection [4]. In 2016, the World Health Organization called for the elimination of HCV infection as a public health threat by 2030. Despite some progress, it was estimated that 57 million people were infected with HCV by 2020, with 300,000 HCV-related deaths occurring annually [5].

There is an important link between HCV infection and the development of HCC, and the exploration of this link can help clinicians detect the risk of HCC as early as possible or select targeted treatments. For instance, Horiike et al. found HCV RNA replication in HCC cells [6]. Transgenic mice expressing the HCV core protein gene displayed early hepatic steatosis, followed by the development to HCC [7]. Given the urgent need for predicting HCV-related HCC, further exploration and elucidation of the specific links between HCV and the development of HCC are necessary. Studying how HCV affects host cell gene expression is a prerequisite for researchs aimed at developing antiviral or HCC risk-reduction therapies. Investigating the connection between the HCV replication cycle and host cell gene expression is a qualitative approach to understanding how HCV infection leads to increased liver cancer risk [1]. One of the significant molecular changes in HCV-infected patients is the dysregulation of specific genes and signaling pathways, often driven by genomic dynamic epigenetic changes. Numerous studies have shown that HCV infection significantly alters the expression of genes related to various cellular functions in liver cells, including cell defense mechanisms (apoptosis, proliferation, and antioxidant responses), cell metabolism (lipid and protein metabolism), and intracellular transport (vesicular transport and cytoskeleton regulation) [8-10]. However, there is a lack of research regarding how the expression of these genes specifically increases the risk of HCC development.

To date, no published studies have summarized the molecular pathways by which HCV infection leads to the pathogenesis of HCC. This article aims to fill this gap, by systematically reviewing the molecular biological links between HCV infection and the risk of HCC development, explore the impact of HCV infection on host cell gene expression, summarize the biological pathways that may be involved in this process, and

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examine their connections with HCC.

2 Results

2.1 HCC Induction Mechanisms

The inducers of HCC typically manifest in diverse forms among different patients, often attributed to variations in underlying medical conditions. Nevertheless, the progression of HCC generally adheres to a specific sequence of disease events, initiating with liver injury caused by various factors and progressing through chronic liver inflammation, fibrosis, cirrhosis, ultimately culminating in liver cancer [11]. The triggering of these mechanisms often begins with the release of Damage-Associated Molecular Patterns (DAMPs) or Pathogen-Associated Molecular Patterns (PAMPs) from damaged or infected cells [11]. These molecular patterns are recognized by Pattern Recognition Receptors (PRRs) on various immune cells, initiating innate immune responses and subsequently leading to inflammatory reactions [11]. Research indicates that the majority of HCC cases (80–90%) are preceded by events of liver cirrhosis [12].

Hepatitis viruses are significant contributors to elevated HCC risk. In a 2018 study, globally, 54.5% of newly diagnosed liver cancer cases were associated with Hepatitis B Virus (HBV) and 21.2% with Hepatitis C Virus (HCV) infections [13]. Apart from increasing the risk through chronic inflammation, the connection between hepatitis viruses and HCC development involves various molecular mechanisms, including participation in cell cycle dysregulation, DNA methylation changes, HBV genome integration into the host genome, chromosomal instability, immune system modulation, epithelial-mesenchymal transition, increased HCC stem cells, and dysregulation of microRNAs [14-15].

2.2 HCV-Induced HCC

The genome of HCV consists of a single-stranded RNA genome totaling 9.6 kb in length. The HCV genome encodes multiple proteins, which are cleaved by cellular and viral proteases after translation, resulting in three structural and seven non-structural proteins [16]. During the infection process, released PAMPs from HCV can activate Pattern Recognition Receptors (PRRs) such as TLR3 and RIG-I on antigen-presenting cells, initiating innate immune responses against HCV replication [15]. T-cell-mediated specific immune responses occur 5-9 weeks after HCV infection, aiming to combat HCV antigens and eliminate the virus [17]. However, in many cases, complete clearance of HCV does not occur, leading to chronic infection, which progresses to liver fibrosis, cirrhosis, and eventually HCC. Immune system dysregulation is considered one of the factors contributing to the outcomes of HCC induced by HCV. HCV infection affects various types of immune cells, especially exerting immunosuppressive effects on the liver immune microenvironment, which decreases the immune cells' anti-tumor activity [18].

Research conducted in Japan and at the Cleveland Clinic suggests that the incidence of HCC in patients with cirrhosis due to non-alcoholic steatohepatitis is significantly lower than in patients with cirrhosis related to HCV. Approximately 4.0% of patients with HCV-related cirrhosis eventually progress to HCC, while a retrospective study of 69 age-matched patients with HCV-related cirrhosis showed a 30.5% incidence of HCC within 5 years [19-20].

2.3 HCC Induction Mechanisms

At the molecular level, the foundation of HCC formation involves mutations in proto-oncogenes and tumor suppressor genes. Furthermore, various epigenetic changes contribute to the molecular etiology of HCC induced by HCV, including DNA methylation, post-translational histone modifications, chromatin remodeling, and non-coding RNA-mediated gene silencing [21].

Animal experiments involving the transfer of these genes into mice have demonstrated enhanced cell proliferation, transformation, and tumor formation [7,21]. These results suggest that the core protein of HCV and other non-structural proteins may play active roles in the development and progression of HCV-related liver disease and HCC. Additionally, HCV has been shown to increase the release of reactive oxygen species (ROS), causing mitochondrial beta-oxidation damage [23-24]. Finally, Genes controlling cell proliferation and apoptosis are also evidence of significant regulation during HCV infection, such as TGF-β, VEGF, Wnt/β-catenin (WNT), cyclooxygenase-2 (COX-2), and peroxisome proliferator-activated receptor alpha (PPARα) [25-29].

HCV infection also leads to changes in the expression of genes involved in metabolism and transport. Genes controlling the synthesis and transport of lipids, phospholipids, and fatty acids show increased expression after HCV infection. For instance, SREBP1, a major transcription factor for lipid synthesis, drives fatty acid synthesis, leading to hepatic steatosis [30]. Host genes regulating vesicle transport between the endoplasmic reticulum, Golgi apparatus, and plasma membrane are also upregulated. The increased expression of RAB6B and RAB33B (controlling retrograde transport from the Golgi apparatus to the endoplasmic reticulum) may promote the accumulation of viral proteins during the formation of replication complexes. Studies indicate an association between elevated mRNA and protein expression of RAB6B in HCC tissues and an immunosuppressive microenvironment, as well as a poorer cancer prognosis [1].

2.4 Pathway Enrichment Analysis Based on HCV-Infected HCC Sequencing Results

This study was based on the transcriptome sequencing

results (GSE20948) of Huh7 liver cancer cells infected with JFH-1 genotype HCV in 2010 by Blackham S and colleagues in the public database (mock-infected cells were used as the control group), and was independently conducted using Reactome and GEO2R tools. Pathway enrichment analysis[1]. After screening all differential genes with at least a 2-fold change in expression and differential expression probability greater than 95% ($P \leq 0.05$), a total of 208 significant differential genes were obtained, as shown in Figure 1 below.

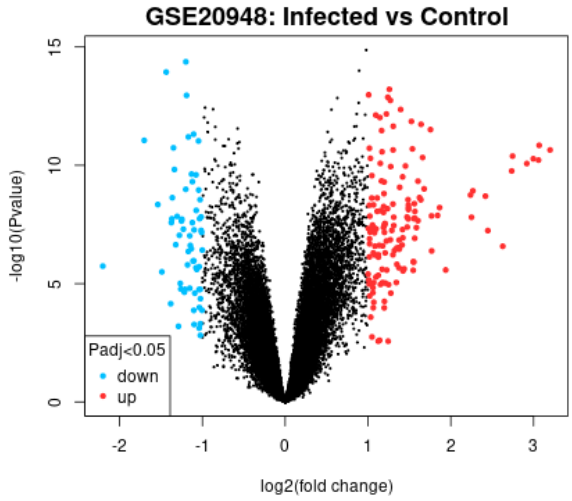

Fig. 1. HCV Infected vs Control volcano map

Figure 2 below shows the top 30 gene pathways as a result of pathway enrichment analysis. In the transcriptome sequencing of HCC cells infected with HCV, numerous differences in gene expression were identified. The expression of Insulin Receptor Substrate (IRS) exhibited a significant upregulation in the HCV-infected group. Notably, the binding of the p85 regulatory subunit of PI 3 kinase to IRS results in the recruitment and activation of the p110 catalytic subunit. Such interactions lead to the cascade activation of PI 3 kinase[31]. Inactive AKT in the cytoplasm binds to PIP3 on the cell membrane, leading to complete AKT activation and subsequent phosphorylation of multiple downstream targets [32]. The PI3K/AKT/mTOR (PAM) pathway is closely associated with the survival, growth, and progression of eukaryotic cells. Additionally, the dysregulation of this signaling pathway is often closely linked to cancer development [33].

Furthermore, an upregulation in the expression of Epidermal Growth Factor (EGF) ligand BTC was observed. The receptor EGFR, exhibiting high affinity for EGF, also showed an upregulation in expression. EREG-related signal transduction plays a crucial role in the normal regulation of physiological stress, inflammation, and vascular genesis [34-35]. Simultaneously, EGF can downregulate the expression of the tumor suppressor E-cadherin through multiple signaling pathways [36].

Several genes related to apoptosis show an upregulation, including the involvement of the PMAIP1 gene in the p53 signaling pathway, the apoptotic transcription factor ATF4, and various downstream genes. Including CHAC1, asparagine synthase (ASNS), PPP1R15A, TRIB3, these genes have been shown to play an important role in organ fibrosis or cancer progression. [37-41].

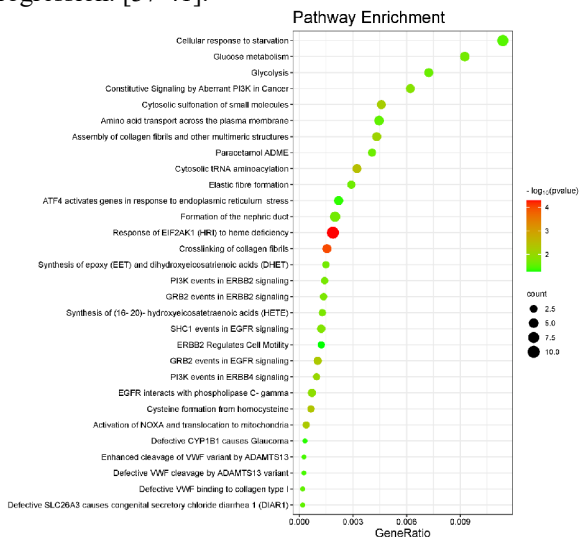

Fig. 2. Top 30 of Pathway Enrichment

In HCV-infected liver cancer cells, there are significant changes in gene expression related to protein expression. Some genes associated with tRNA synthesis also show upregulation. Studies have found a significant correlation between increased GARS expression in HCC tissue and poorer overall and disease-free survival [42-43]. Viruses depend on the reprogramming of tRNA groups in host cell protein production, research has focused on using tRNA for the diagnosis of hepatitis viruses[44]. Sulfotransferases (SULT) involved in the cytoplasmic sulfation process also exhibit high expression, studies suggest that SULT can regulate cancer progression by modulating cancer cell metabolism. Meanwhile, genes involved in type I collagen and elastic fiber synthesis show decreased expression. Liver fibrosis is characterized by extensive deposition of extracellular matrix (ECM) proteins, especially type I collagen. The downregulation of these genes suggests a counterintuitive progression, requiring further exploration. Lastly, post-infection, there is an increase in the expression of genes involved in protein sorting and vesicle transport, including those regulating protein transport between the endoplasmic reticulum and the Golgi apparatus, and genes associated with vesicle transport between membranes.

3 Discussion

The review of existing studies shows that HCV infection significantly alters the expression of genes related to host cellular immune responses, apoptosis, metabolism, protein synthesis, and intracellular trafficking [1], [23-29]. The validation based on pathway enrichment analysis also showed molecular mechanisms that were highly consistent.

Firstly, HCV infection in this study markedly alters the expression levels of the PI3K/AKT/mTOR (PAM)

pathway, and its widespread activation is initiated by IRS-mediated signaling. Aberrant expression of the PI3K/AKT/mTOR (PAM) pathway has been widely demonstrated to be closely associated with cancer development[41]. Genes associated with apoptosis are also significantly regulated during HCV infection, including genes related to P53 signaling and apoptotic transcription factors. This response may correspond to the cytotoxic effects of the host cell immune system in response to viral infection. The upregulation of apoptosis-related pathways induced by HCV may also mediate the death of liver cells, exacerbating the progression of liver fibrosis. Notably, protein synthesis and genes associated with tRNA synthesis show substantial changes post-HCV infection. Sulfotransferases (SULT) involved in cytoplasmic sulfation processes exhibit marked upregulation. Although research on how HCV is involved in this process is yet to be published, it can be inferred that these changes in protein expression are related to the production of HCV viral proteins within host cells, a mechanism reported in studies on other viruses like HIV . Studies using tRNA as a diagnostic target for viral infections are also underway. Lastly, genes regulating the transport of proteins between the cytoplasm exhibit significant expression changes. It can be speculated that these genes are closely related to the entry and exit of the virus in host cells, as well as the synthesis of viral proteins. The relationship between these changes and the formation of HCC has not been proposed in existing viewpoints.

4 Conclusion

This study summarizes the connection between gene expression profile changes after HCV infection and the development of HCC. The results suggest that HCV-induced alterations in gene expression primarily focus on cellular stress/inflammatory responses, pathways related to cell growth and apoptosis, and protein synthesis. Further research on the molecular level of these gene expression patterns can expand our understanding of the pathological changes induced by HCV in the liver, providing avenues for precise diagnosis of HCV-related HCC. There are still limitation in this study. Due to shortage of resources, this study can only speculate on the mechanism of HCV-induced HCC from the gene expression level. Future studies should further integrate the molecular mechanisms of HCV infection with changes in gene expression in host cells at the protein level, evaluating potential diagnostic/therapeutic nodes within these molecular mechanisms. Finally, based on possible findings in these future studies, some gene-targeted therapies, or active drug monomers, will be developed to prevent or treat the development of HCC associated with HCV infection.

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