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REVIEW ARTICLE

Biochemical overview of the recent findings on the correlation between viral hepatitis and its related hepatocellular carcinoma

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Abstract

As one of the most common primary liver cancers, hepatocellular carcinoma (HCC) usually occurs in the presence of inflammation. Compared to other risk factors such as alcohol abuse, aflatoxin, and obesity, virus-induced hepatitis can be effectively prevented by vaccines. For the past several decades, HCC has been believed to be closely related to viral infections although no comprehensive mechanism was established regarding the contribution of viral hepatitis toward HCC. Recent studies have shown that viral infection plays multiple roles in the process of carcinogenesis by causing an increase in genomic instability, cancer-promoting genetic mutations, signal pathway interruption, and tumor suppressor gene inhibition. Sorafenib has become a novel option for HCC patients, especially those who are in advanced disease stage for which conventional treatment methods are not recommended. Future studies should focus more on novel targeted drugs which can be adopted as alternatives to sorafenib or as second-line drugs after the failure of sorafenib

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According to Dr. Jeffrey Stanaway, worldwide, 1.45 million lives were lost to viral hepatitis in 2013, which was significantly greater than the 0.89 million deaths in 1990. Among all viral hepatitis-related morbidity cases, the prevalence of hepatitis C infection-related morbidity was the highest [1]. It has been well known that hepatitis-related mortality is influenced by geographical variation; Oceania, western-sub-Saharan Africa, and central Asia have the highest mortality rate. Not surprisingly, East Asia and South Asia have the highest absolute numbers because of their large population [2]. Hepatitis-related cirrhosis is often observed in viral hepatitis patients as the sequential progression. Scientists have long believed that there is a close association between hepatitis-related cirrhosis and hepatocellular carcinoma (HCC) although there is no comprehensive understanding yet. HCC is the most common primary liver cancer with a complex etiology and has mechanisms that make early clinical detection and treatment of this disease challenging. As one of the most critical risk factors for HCC, viral hepatitis and hepatitis-related cirrhosis deserve more attention in future studies. The aim of this paper was to review biomolecular characteristics of viral hepatitis and some recently proposed findings with regard to the correlation between viral hepatitis and its associated HCC along with discussing potential treatment and future perspectives.

Pathophysiology of viral hepatitis and viral hepatitis-related responses

Molecular biology of HBV and HCV infections

The four overlapping reading frames are encoded by HBV viral genome which includes the following ORFs: S, C, P, and X. The viral surface proteins and HBsAg are encoded by S ORF. The core gene (C) is responsible for the synthesis of either hepatitis B core antigenor (HBcAg) hepatitis B e-antigen (HBeAg). The large protein polymerase (pol), which facilitates viral replication, is encoded by P ORF. The viral X ORF encodes HBxAg which is vital for signal transduction, transcription activation, DNA repair, and inhibition of protein degradation [3]. HBV invades the cell by binding to its receptor, namely

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the bile acid pump sodium-taurocholate co-transporting polypeptide A (NTCP1), on the cell surface and releases its DNA into the target cell nucleus. After converting to a cccDNA minichromosome, HBV transcription can be performed using cccDNA as the template [4]. The transcription is driven by promoters and regulated by transcription factors, and reverse transcription is completed in the presence of viral polymerase using pre-genomic RNA as the template. The relaxed circular DNA (rcDNA) of the virus is formed after a complementary strand is created. Binding with viral polymerase enables rcDNA packaging and new virus formation [5].

Because Hepatitis C virus is an RNA virus different from HBV, the positive stranded RNA of HCV is cleaved into 10 proteins by its host cell and viral proteases after its translation [core protein, envelope 1 and 2 (identified as structural proteins, and p7 as well as NS2, NS3, NS4A, NS4B, NS5A, and NS5B (classified as non-structural proteins)] through a cap-independent mechanism mediated by HCV IRES. Similarly, the replication of HCV is carried out by the synthesis of positive and negative strands of HCV RNA, which serve as the template. The replication is mainly regulated by cis-elements. Additionally, some long-range RNA-RNA interactions (LRIs) and liver-specific microRNA-122 (miR-122) play significant roles in viral RNA translation and replication [6].

It has long been believed that chronic hepatocytic injury is induced by immune cell activity in cases of both HBV and HCV infections rather than via a cytopathic effect. In both HBV and HCV infections, liver injury is usually caused by the inflammatory response. Hepatocytes release proinflammatory cytokines such as TNF-á, IL-6, IL-1, and IL-18, and initiate the accumulation of granulocytes, mononuclear phagocytes, and macrophages at the site of inflammation as well as facilitate the elimination of the invading virus. Because of the over-reactive immune response against the virus, several hepatocytes can accidentally get injure during the inflammation process [7]. In patients without an efficient adaptive immune system, acute viral infection can develop into a chronic infection. During the prolonged process of inflammation, multiple types of hepatic cells are involved. The hepatic stellate cells (HSCs) are normally located in the space between hepatocytes and endothelial cells. The quiescent HSC mainly plays a role in vitamin A storage. When HSCs are activated during the inflammatory response, extracellular matrix and collagen production by these cells is triggered by releasing TGF-â which is a strong inducer of fibrogenesis. Moreover, the pattern of adhesion molecule expression in endothelial cells is altered during chronic inflammation [8]. Similar to endothelial cells and HSC, Kupffer cells also release cytokines and chemokines during inflammation and exhibit changes in the expression of adhesion molecules.

Immunologically, cytotoxic T cells are involved in viral elimination by recognizing the viral antigens presented on the host cell surface, and this mechanism is responsible for the clearance of a majority of viruses during an acute infection. Other immune cells also play roles in the inflammation process. The pit cells (a type of NK cells) respond to inflammation as well. They produce IFN-ã, TNF-á, and cytokines to destroy the abnormal cells (malignant or infected cells). Neutrophils are attracted by cytokines released from Kupffer cells and hepatocytes and migrate from other parts of the body to hepatic tissues. The net result is to increase inflammation and intensify inflammatory injury caused to the liver [9].

During inflammation, multiple inflammatory factors are released by cells. Moderate cell necrosis and extracellular matrix damage will follow inflammation and cell injury. Consequently, fibrogenesis occurs for repairing the injured tissues and replacing them with granulation tissues. Once reaching an advanced level, fibrogenesis progresses to cirrhosis, which is a later stage of fibrosis; during this stage, regenerative nodules and fibrotic bands are formed in the perisinusoidal space to give the liver a “bumpy” and stiff texture [10].

Involvement of molecular pathogenesis in oncogenesis

Cirrhosis is one of the most important risk factor of HCC and exhibits a close correlation with HCC, which is the cancer responsible for the highest morbidity rate among all primary liver malignancies worldwide. Among 80%–90% of HCC cases, HBV or HCV infection occurs, and the 5-year cumulative risk of HCC development in virus-induced cirrhosis patients varies from 5% to 30% [11]. According to Fattovich, the estimated incidence rate of HCC in East Asia among inactive HBV carriers is 0.2 per 100 person-years and 0.6 per 100 person-years among chronic HBV infection patients without cirrhosis. Additionally, the incidence of HCC significantly increases in chronic HBV infection patients with cirrhosis [12]. Moreover, the positive correlation between cirrhosis severity and HCC incidence has been proved by another cohort study conducted in Taiwan from 1997 to 2011 [13]. The inflammatory response, oxidative stress, and fibrosis may contribute to hepatocyte transformation and HCC development. However, the links between virus-induced hepatitis and HCC have not been clearly understood. Thus far, several mechanisms have been suggested for the initiation of HBV-induced HCC. First, HBV-induced chronic inflammation and hepatocyte regeneration could cause genetic alterations. Second, genomic instability and alteration of cancer-related gene expression can be induced by HBV integration; this

would finally lead to hepatocarcinogenesis. In a previous study, HBV integration events were observed in some common host genes including KMT2B, FN1, and TERT [14]. In recent years, multiple roles of HBV-X protein in the process of oncogenesis have been widely recognized. HBx is involved in cellular signal pathways, transcription regulations, cell cycle mediation, DNA repair, apoptosis, and genetic stability through interactions with host factors [15]. However, the mechanism of HCC induction by HBV remains unclear. The development of HBV-induced HCC might be owing to the combined actions of all mechanisms discussed previously. In mice models, the expression of HBx in liver tissues results in increased susceptibility to hepatocarcinogens [16]. HBx can bind to p53 and inhibit its activity as a tumor suppressor through a complicated mechanism. HBx can also bind to NF-êB (nuclear factor) and promote HCC cell invasion and metastasis [17]. Additionally, HBx also plays a role in the regulation of histone modification. For instance, HBx can interfere with gene transcription and stimulate tumorigenesis through interaction with CBP/p300 histone acetyl-transferase complex, and this phenomenon can often be observed in HBV-related HCC [18].

HBV and HCV infections are reportedly related to elevated levels of â-catenin, which is an important part of Wnt/â-catenin signaling pathways and considered to be significant in cancer development. An abnormality in the Wnt/â-catenin signaling pathway increases the incidence of cancer [19].

Furthermore, some mutations seem to be closely related to viral infection and HCC. In HBV virus, mutations can occur in multiple gene regions because HBV reverse transcriptase does not exhibit a proofreading function. Pres/S, preC/C, polymerase/reverse transcriptase, and X ORF gene sites are the most common regions undergoing mutations that are closely related to the development of HCC [20]. Somatic mutations in protein coding genes such as Wnt (CTNNB1) and TP53 are most commonly encountered during HCC infection, and mutation is observed more frequently in TP53 during HBV infection [21]. TERT promoter mutations were prevalent in 59% of the tested human HCC samples, and this mutation was reportedly involved in malignantly transforming hepatocellular adenoma into HCC [22]. Moreover, the frequency of mutation in the TERT promoter increased with an increasing grade of dysplastic nodules (low grade too high grade) and peaked during the early stages of HCCs. Besides, TERT promoter mutation is recognized as the earliest somatic alteration and believed to be a novel predictive biomarker for oncogenesis stemming from cirrhosis [23]. Moreover, telomerase promoter mutations can cause telomerase overexpression and telomere shortening. Telomere shortening causes cell senescence or apoptosis and decreases the regeneration ability of liver cells, consequently resulting in fibrosis and cirrhosis, which is a precursor to HCC [24].

Dysregulated DNA methylation is another factor for HCC in chronic HBV and HCV infections. Dysregulation of DNA methylation includes hypomethylation and hypermethylation. Hypomethylation influences structural-nuclear function, and hypermethylation can lead to the inhibition of tumor suppressor genes. Specifically, methylated genes which involved signaling pathways such as RAS/RAF/ERK and Wnt/â-catenin pathways are observed more often in HCV-induced HCC. Methylation of GSTP1 and E-cadherin promoters preferentially occurs in HBV-induced HCC [25].

Unlike HBV infection, HCV infection does not integrate into the host genome. However, inflammation, steatosis, oxidative stress, and progressive fibrosis in HCV infection ultimately lead to HCC development. HCV infection indirectly triggers hepatocarcinogenesis. Moreover, it directly affects physical processes including metabolism, DNA repair, and apoptosis by altering cellular pathways [26]. HCV can contribute to the breaking of double-stranded DNA, thereby increasing the possibility of gene mutations. Such mutations can be found in immunoglobulin genes, BCL-6, TP53, and oncogene CTNNB1 (encodes â-catenin in WNT pathway) [27]. HCV-infected cells showed elevated â-catenin levels, although its significance is not clear yet [28]. E1, E2, NS3, and NS5 as core proteins of HCV are thought to be crucial for tumorigenesis because they modulate cell signaling by interacting with several cellular proteins [29]. Additionally, as a viral product protein, HCV capsid is involved in the process of cell proliferation and modulates cellular gene functions. A previous study has reported that transfection of the *ras* oncogene causes rat embryo fibroblasts to be transformed into a malignant phenotype by the human *c-myc* promoter gene in the presence of HCV capsid [30]. Moreover, Nishiguchi et al emphasized the correlation between HCV infection and HCC in the study investigating the effects of IFN-á on patients with HCV and cirrhosis. In that study, only 4% of the patients in the IFN-á group develop HCC as opposed to 38% of patients in the placebo group [31].

Additionally, HCC occurrence is not correlated only with viral infection. Cirrhosis itself is also an independent factor which increases the risks of HCC. A noteworthy link between HCC incidence and severity of virus-induced cirrhosis is proved by the data collected from 1997–2011 in Taiwan. It has been revealed that cirrhosis patients with other complications have a significantly higher risk of HCC compared with cirrhosis patients without any complications [32]. Other than that, underlying cirrhosis in HCC patients makes the disease treatment more challenging and also significantly reduces the survival rate because of its association with several

complex and critical complications. The irreversibility and complicated complications coming along with cirrhosis gives a moderate survival rate of patients.

Discussion and future perspectives

Currently, the first-line treatment for patients with solitary tumors is hepatic resection (with well-preserved liver function). Specifically, normal serum bilirubin levels with either an HVPG of ≤ 10 mm Hg or a platelet count of $\geq 100\times10^9$/L. For patients with poorer liver function and at a Child–Pugh stage A or B, local ablation is suitable for tumors measuring < 3 cm in size. Other than that, liver transplantation is the optimal choice for patients with single tumors of size ≤ 5 cm or up to three tumors measuring ≤ 3 cm in size. For patients with compensated liver disease and large or multifocal HCC without vascular invasion or extrahepatic spread, transarterial chemoembolization (TACE) is considered prior to other alternatives [33].

Nowadays, the multi—kinase inhibitor sorafenib has become the standard therapy in advanced HCC and Child–Pugh class A according to the current guidelines. However, novel drugs are needed for advanced HCC patients as alternatives to sorafenib failure. Antibody against cell death protein (PD-1) as an immune checkpoint inhibitor showed an overall response rate of 19% in HCC patients in a phase I study reported in 2015. Anti-PD-1 (nivolumab) achieves the goal of tumor attack through T cell re-storage and prevents cancer immunotolerance [34]. Besides, lenvatinib has been discovered to be an effective angiogenesis inhibitor which primarily suppresses tumor growth by blocking vascular endothelial growth factor receptors (VEGFR1, VEGFR2, VEGFR3), fibroblast growth factor receptors (FGFR1, FGFR2, FGFR3, and FGFR4), KIT, and RET [35].

Furthermore, recent studies have identified that miR-214 is a tumor suppressor, which significantly inhibits on cell growth, cell proliferation, and colony forming ability as well as decreases the levels of â-catenin protein and its downstream proteins including Cyclin D1, c-Myc and TCF-1 [36]. This finding may shed light on a novel perspective for future HCC treatment.

Conclusion

Although HCC is a disease caused by many factors and characterized by its great difficulty in treatment, viral infection is the controllable factor that can be prevented effectively through vaccination and health education; these strategies can greatly lower the incidence of virus-induced HCC, thereby decreasing the mortality. Mechanisms underlying the development of HCC caused by the association of viral infection (HBV and HCV) with cirrhosis have not been clearly understood. However, the effects of viral infection on gene mutations and signal pathways are widely recognized. Moreover, the vital role of HBx in oncogenesis is has been recently proved. Traditional options of HCC treatment are mainly focused on hepatic resection, local ablation, and TACE according to concrete states of disease. Sorafenib is the only targeted drug recommended by the current guidelines for HCC treatment.

Conflict of interest

The authors confirm that this article has no conflict of interest.

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