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TOPIC HIGHLIGHT

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Hepatitis B virus infection and intrahepatic cholangiocarcinoma

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Abstract

Intrahepatic cholangiocarcinoma (ICC) is a devastating malignant tumor arising from the peripheral intrahepatic bile duct epithelium. The incidence and mortality of ICC is markedly increasing over the past two decades worldwide, though the cause for this rise in incidence is unclear, thus intensifying the search for alternative etiological agents and pathogenetic mechanisms. Hepatolithiasis, primary sclerosing cholangitis, parasitic infection (*Opisthorchis viverrini* or *Clonorchis sinensis*), fibropolycystic liver disease, and chemical carcinogen exposure are thought to be the risk factors for ICC. Nevertheless, the majority of ICC patients do not have any of these risk factors, and none of the established risk factors can explain the recent increasing trend of ICC. Therefore, identifying other risk factors may lead to the prevention and early detection of ICC. Chronic hepatitis B virus (HBV) infection is the predominant cause of hepatocellular carcinoma in HBV-endemic areas. This review discusses the evidence implicating chronic HBV infection as a likely etiology of ICC and the pathogenetic mechanisms that might be involved.

Key words: Hepatitis B virus; Intrahepatic cholangiocarcinoma; Epidemiology; Etiopathogenesis

Core tip: Intrahepatic cholangiocarcinoma (ICC) is a devastating malignant tumor. Its incidence and mortality is increasing drastically over the past two decades worldwide, though the cause for this rise in incidence is unclear. The etiology and carcinogenesis of ICC remain inconclusive. Recent studies suggest that hepatitis B virus (HBV) infection plays an important etiological role in ICC development. HBV-associated ICC holds many clinicopathological similarities with HBV-associated hepatocellular carcinoma (HCC), and HBV-associated ICC patients may have a better prognosis than ICC patients without HBV infection. HBV-associated ICC and HBV-associated HCC may share a common disease process for carcinogenesis, through a similar long-term inflammatory carcinogenic process, and both possibly arise from hepatic progenitor cells.

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INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC), a bile duct carcinoma arising from either the second-order or more peripheral branches of the intrahepatic bile duct[1], occurs much less common than hepatocellular carcinoma (HCC). Although the incidence of ICC is very low, it is

the second most common type of primary liver cancer behind HCC, accounting for 10%-15% primary liver cancers and its incidence and mortality are increasing worldwide[2-7]. However, the etiology of ICC is largely unknown. Although several potential risk factors have been established, including chronic biliary tract diseases (*i.e.*, primary sclerosing cholangitis, hepatolithiasis, and Caroli's disease), parasitic infestation of the biliary tract by endemic *Opisthorchis viverrini* and *Clonorchis senensis*[8-10], and nonbiliary diseases such as heavy alcohol use, obesity, nonalcoholic fatty liver disease, chronic hepatitis C, and cirrhosis[11-15], ICC occurs mostly in the absence of these established etiological factors, and none of these risk factors can explain the recent increasing trend of ICC. Therefore, identifying other risk factors may lead to the prevention and early detection of ICC.

HBV is the prototype member of a family of small, enveloped DNA viruses called hepadnaviruses. Chronic HBV infection is the most common cause of HCC worldwide: more than 50% of global HCC cases and 70%-80% of HCC cases in highly HBV endemic regions, such as eastern Asia and sub-Saharan Africa, are attributable to HBV[16-18]. The etiopathogenesis of ICC was once considered to be independent of the presence of chronic HBV infection or HBV-associated cirrhosis, but some recent epidemiological data indicate a causal role for chronic HBV infection or HBV-associated cirrhosis in the development of ICC, particularly in HBV-endemic areas[19-25]. Nonetheless, the role of HBV and its carcinogenesis in the etiology of ICC remain unclear. This review focuses on the evidence and possible pathogenic mechanisms in support of a role for HBV in the etiology of ICC.

EPIDEMIOLOGICAL STUDIES OF ICC-ASSOCIATED HBV

Over the past two decades, there have been 15 epidemiological studies (13 case-control and two cohort studies) examining the relationship between HBV and ICC (Table 1). Of these, four were from China[20-22,26], four from the United States[11-13,27], two from China Taiwan[28,29], two from Japan[15,23], two from South Korea[19,30], and one from Italy[24].

Of the 13 case-control studies, two were population based, all from the United States; the eleven remaining studies were hospital based. Seven found a statistically significant positive association between serum hepatitis B surface antigen (HBsAg) and ICC (range of individual ORs, 2.3-9.67)[13,19-22,26,28], whereas the remaining studies did not[11,12,15,24,27,30]. Six of the seven studies reporting a positive correlation were performed in both regions of high prevalence of hepatobiliary cancers (*i.e.*, China, Japan, and South Korea), with one study in a region of lower prevalence (the United States). One study revealed a positive and significant association for patients with anti-HBc (hepatitis B core antibody), though the results did not reach significance for those with serum HBsAg alone[12]. Age and gender adjustments were reported in all the 13 case-control studies.

Two cohort studies have been conducted. The first retrospective cohort study from the province of Osaka in Japan included 154814 apparently healthy individual blood donors, aged 40-64 years at the time of blood donation in the period 1991-1993[23]. The average observation period was 7.6 years. Incident ICC cases were identified by linking the blood-donor database to the records in the population-based cancer registry for the province. There were 11 incident ICC cases during follow-up, with an incidence rate of 0.88 per 100000 person-years. Compared to those who tested negative for both HBsAg and hepatitis C antibody (anti-HCV), those who tested HBsAg-seropositive had a significantly higher risk for ICC (HR = 8.56; 95%CI: 1.33-55.20). These results suggest that HBV infection is independently associated with ICC development.

The second cohort study was from Taiwan[29] and included 1782401 pregnant Taiwanese women whose HBV serostatus was obtained from the National Hepatitis B Vaccination Registry. Newly diagnosed ICCs were ascertained through data linkage with the National Cancer Registry. Eighteen cases of ICC were recorded during a mean follow-up period of 6.9 years. The incidence rates of ICC were 0.09 and 0.43 per 100000 person-years among women who were HBsAg-seronegative and HBsAg-seropositive, respectively, showing an age-adjusted HR (HRadj) of 4.80 (95%CI: 1.88-12.20). The study also suggests that chronic HBV infection is associated with an increased risk of ICC.

META-ANALYSES OF ICC-ASSOCIATED HBV

To better elucidate a possible association between HBV and ICC, three recent meta-analyses have been conducted, with two focusing on risk factors for ICC alone. All of three studies were published in 2012; of these, two were from China[31,32] and one from The Mayo Clinic, United States[33].

The first meta-analysis by Li *et al*[31] included a total of 18 studies, 16 case-control studies and 2 cohort studies. The pooled risk estimate of all of the studies showed a statistically significant increased risk of cholangiocarcinoma among individuals with HBV infection [RR = 2.66; 95%CI: 1.97-3.60]; compared to those without HBV infection, persons with HBV infection had an increased risk of ICC (RR = 3.42; 95%CI: 2.46-43.74). In a subgroup analysis of HBV infection and risk of ICC, the pooled risk estimate of studies in Asians (RR = 3.63; 95%CI: 2.56-5.13) was higher than that in non-Asians (RR = 1.93; 95%CI: 0.78-4.76). A Begg funnel plot and Egger test revealed no evidence for publication bias.

The second meta-analysis was conducted by Palmer *et al*[33] from the United States. Nine case-control studies investigating hepatitis B as a risk factor from regions of both high and low prevalence of hepatobiliary cancers were selected. All of the studies, except those evaluat-

Table 1 Characteristic of case-control studies of hepatitis B virus infection and intrahepatic cholangiocarcinoma risk

Study	Study design	Dates	Cases		Controls		Source	OR (95%CI) for HBV infection (serum HBsAg, unless stated otherwise)
			Total	With exposure	Total	With exposure		
Donato *et al*[24], 2001	Case-control	1995-2000	26	13%	824	5.5%	Hospital	2.7 (0.4-18.5)
Yamamoto *et al*[15], 2004	Case-control	1991-2002	50	4%	205	2%	Hospital	1.84 (0.34-10.11)
Shaib *et al*[11], 2005	Case-control	1993-1999	625	0.2%	90834	0.2%	Population	0.8 (0.1-5.9)
Choi *et al*[30], 2006	Case-control	2003-2004	51	7.8%	51	9.8%	Hospital	0.8 (0.2-3.02)
Shaib *et al*[12], 2007	Case-control	1992-2002	83	1.2%	236	0.4%	Hospital	2.9 (0.1-236.8) anti-HBc 28.6 (3.9-1268.1)
Lee *et al*[19],2008	Case-control	2000-2004	622	13.5%	2488	5.0%	Hospital	2.3 (1.6-3.3)
Zhou *et al*[22], 2008	Case-control	2004-2006	312	48.4%	438	9.6%	Hospital	8.9 (5.97-13.19)
Tao *et al*[20], 2009	Case-control	1998-2008	61	27.9%	380	5.0%	Hospital	7.3 (3.1-17.2)
Lee *et al*[28],2009	Case-control	1991-2005	160	37.5%	160	13.8%	Hospital	4.99 (2.78-8.95)
Tanaka *et al*[23], 2010	Cohort	1991-1993		9.08%[1]		0.66%[2]	7.6 yr of follow-up	8.56 (1.33-55.20)
Zhou *et al*[21], 2010	Case-control	2003-2006	317	48.6%	634	6.6%	Hospital	9.67 (6.33-14.77)
Peng *et al*[26], 2011	Case-control	2002-2009	98	31.6%	196	12.8%	Hospital	2.75 (1.27-5.95)
Fwu *et al*[29], 2011	Cohort	1983-2000		0.43%[3]		0.09%[4]	6.91 yr of follow-up	4.80 (1.88-12.20)
Welzel *et al*[13], 2011 United States	Case-control	1993-2005	743	1.5%	195953	0.2%	Population	3.1 (1.43-6.58)
Chaiteerakij *et al*[27], 2013	Case-control	2000-2010	612	3.0%	594	3.0%	Hospital	

[1]Incidence rate of intrahepatic cholangiocarcinoma (ICC) per 100000 hepatitis B surface antigen (HBsAg)-seropositive person-years; [2]Incidence rate of ICC per 100000 HBsAg and anti-hepatitis C virus-seronegative person-years; [3]Incidence rate of ICC per 100000 HBsAg-seropositive woman-years; [4]Incidence rate of ICC per 100000 HBsAg-seronegative woman-years. HBV: Hepatitis B virus; Anti-HBc: Hepatitis B core antibody.

ing cirrhosis, diabetes, and obesity, exhibited significant heterogeneity. Of these, the authors excluded one study in which HBV infection was identified solely on the basis of anti-HBc positivity. In the other studies, HBV infection was defined by the presence of HBsAg (6 studies), HBV DNA in serum (1 study), or by ICD9 codes (1 study). The study data collection ranged from 1991 through 2008, encompassing a total study population of 294828 patients. The meta-analysis indicated that the presence of HBV was associated with a combined OR of 5.54, with a 95%CI of 3.19-9.63 for ICC. Five of the analyzed studies were performed in high-prevalence regions in Eastern nations, such as Japan, South Korea, and China, whereas three studies were from Western nations in low-to-intermediate prevalence regions, such as the United States and Italy. A separate analysis of these two groups did not reveal any significant difference between the two regions.

The final meta-analysis by Zhou *et al*[32] included a total of 16 studies: 3 cohort and 13 case-control studies. The combined risk estimate of all the studies showed a statistically significant increased risk of ICC incidence with HBV infection (OR = 3.17; 95%CI: 1.88-5.34). With regard to the case-control studies alone, the combined OR of HBV infection was 2.86 (95%CI: 1.60-5.11), whereas the OR of HBV was 5.39 (95%CI: 2.34-12.44) for the cohort studies. This meta-analysis further suggested that HBV infection is associated with an increased risk of ICC.

In conclusion, there is a large amount of epidemiological data in recent strongly supporting an association between HBV infection and ICC, particularly in HBV-endemic regions.

PATHOLOGICAL STUDIES OF ICC-ASSOCIATED HBV

To date, only four studies have been conducted to investigate the presence of HBV DNA, genes, and proteins in ICC pathology specimens; three of the four studies were from China and one from the United States. The earliest study was from China[34], published in 1998, and analyzed 30 ICC samples and the surrounding hepatic tissue. HBV antigen expression, including HBsAg, hepatitis B core antigen (HBcAg), hepatitis B X antigen (HBxAg), pre-S1, and pre-S2, was found in 12 of the 30 ICC cancerous tissues. Eight cases showed HBxAg positivity, five were positive for pre-S1, and five were positive for pre-S2; in contrast, no case was positive for HBsAg or HBcAg. Five types of probes were applied to detect S gene, pre S gene, C gene, X gene and HBV DNA in the cancerous tissues: HBV DNA was detectable in 23 of the 30 ICC cancerous tissues, and the HBV X (HBx) gene, pre S gene, S gene, and C gene were identified in 20, 13, 12, and 9 cases, respectively.

A second study evaluated 11 ICC samples, with surrounding tissue present for 10 cases[35]. Serum HBsAg was detected in 2 cases, and the 9 remaining cases included no data for serum HBsAg. Although no HBV DNA was detected in the 13 control cases that were seronegative for HBsAg, HBV DNA was detected in 2 (18.2%) of the 11 ICC livers. In 1 case, HBV DNA was detected in both the normal and tumor tissues, whereas it was detectable only in the normal tissue in the other case. In 1 case, the surface, core, and X genes and cccDNA were detected; only the surface and core genes and cccDNA were detectable in the other case. In both

cases, the copy numbers of HBV DNA were low, with less than 200 genome copies per microgram of total liver DNA. Nucleotide sequencing of the surface gene amplicon (672 bp) revealed infection with HBV genotype A in both cases, also revealing marked differences between the 2 cases of HBV genotype A.

The third study included 23 ICC cases between January 2002 and December 2008[36]. HBsAg seropositivity was found in 52.2% (12/23), HBV DNA (X region) in the liver was detectable in 34.8% (8/23), and HBV antigens in liver tissues were detected by immunohistochemistry in 30.4% (7/23). Only HBsAg was detectable, though one case was positive for HBcAg; both HBsAg and HBcAg were detectable in 5 cases. All cases with detected viral protein were also positive for HBV DNA.

The final study[37] included 45 ICC cases, all coexisting with HBV infection. The data collection ranged from December 2008 to April 2009. HBV infection was confirmed by the serological detection of HBsAg, and HBx protein expression was found in 70.4% (38/54) of the paraffin-embedded ICC tissue specimens.

The finding of HBV DNA, genes, HBV antigens, and HBx protein in ICC tissue specimens further supports a potential role for HBV infection in the development of ICC. Moreover, the finding of high rates of HBx protein suggests that HBx may play an important role in the pathogenesis of ICC, though it should be noted that all studies involved relatively small cohorts.

HBV AND CLINICOPATHOLOGICAL FEATURES OF ICC

For further elucidating the role of HBV in the development of ICC, some recent studies have investigated the impact of HBV infection on the clinicopathological features of ICC. A study from Taiwan showed that the mean age of hepatitis B-associated ICC patients (56.4 ± 11.1 years) was 9 years younger than that of hepatitis C-associated ICC patients (65.6 ± 9.17 years), similar to that observed in HCC. Moreover, the profiles of the age distribution between ICC and HCC patients with hepatitis B were essentially consistent[28]. The data from our previous study also demonstrated that the age distribution profile was nearly identical between seropositive-HBsAg ICC patients and HBV-associated HCC patients. We also found that, compared to seronegative-HBsAg ICC patients, seropositive-HBsAg ICC patients were younger, more frequently male (similar to HBV-associated HCC), had a higher incidence of abnormal aminotransferase and serum alpha-fetoprotein (AFP) levels, histological inflammation and cirrhosis, right lobe focus, poor tumor differentiation, tumor encapsulation and microvascular invasion, and had a lower incidence of abnormal serum carbohydrate antigen 19-9 (CA19-9) levels and lymphatic metastasis. These results suggest that HBV-associated ICC shares many clinicopathological similarities with HBV-associated HCC and were further supported by other studies[26,38].

On the basis of gross morphological features, ICC can be classified into three subtypes: mass forming, periductal infiltrating, and intraductal[39]. These different growth patterns suggest that ICCs are heterogeneous tumors having different cells of origin and different pathogeneses. It is likely that periductal and intraductal tumors arise from the malignant transformation of epithelial cells lining the larger bile ducts, whereas the mass-forming type arises from smaller bile ducts or bipotential hepatic stem cells within portal areas. Therefore, etiologies involving distinct molecular pathways may be associated with the subtypes of ICC. Indeed, ICCs associated with hepatolithiasis and *Clonorchis sinensis* infection are nearly always found to have the intraductal growth pattern[40,41], whereas viral hepatitis-associated ICCs are frequently found to have the mass-forming growth pattern[38,42]. ICC can also be classified into two subtypes on the basis of histological features: the cholangiolar and bile duct subtypes. Yu *et al*[42] found that the cholangiolar subtype was more strongly associated with viral hepatitis (seropositive for HBsAg and/or anti-HCV) than the bile duct subtype (OR = 2.71; $P = 0.008$), further supporting aforementioned theory of different etiologies involving distinct molecular pathways in ICC development.

N-cadherin (also referred to as CDH2) is a calcium-binding, single-pass transmembrane cell adhesion molecule and a recently identified marker of hepatobiliary tumors[43]. N-cadherin is expressed in a membranous pattern in hepatocytes, interlobular bile ducts, and ductular reactions but not in extrahepatic and large intrahepatic bile ducts. N-cadherin is more frequently expressed in peripheral than in hilar cholangiocarcinomas[43], which suggests that N-cadherin is more likely to be expressed in tumors differentiating toward small bile duct morphology. A subsequent study found that N-cadherin is an immunohistochemical marker strongly associated with hepatitis virus infection (seropositive for HBsAg and/or anti-HCV) (OR = 5.06; $P = 0.0002$); the prevalence of viral hepatitis in ICC patients with N-cadherin-positive intrahepatic cholangiocarcinoma was 75%, whereas that in N-cadherin-negative ICC patients was only 37%[42].

CK19, a member of the type 1 group of cytokeratins with a molecular weight ranging from 40 to 56 kDa[44], is normally expressed in ductal epithelium (bile ducts, pancreas, and renal collecting tubules) and in the mucosa of the gastrointestinal tract (GIT)[45]. The use of CK19 immunohistochemistry in diagnostic pathology has mainly been implemented to confirm epithelial immunophenotypes in potentially undifferentiated tumors or to establish a biliary/pancreatic/renal ductular origin, usually as part of a larger panel of markers. With regard to tumors, CK19 is expressed in squamous carcinomas of the head and neck, HCCs and more than 50% of renal cell carcinomas[46]. Most GIT adenocarcinomas are CK19 positive, including intrahepatic cholangiocarcinomas[21,47]. Recent studies have reported that CK19 expression occurred more frequently in ICC in the absence of HBV infection compared to HBV-associated ICC (88.96% *vs*

99.38%)[21,47].

HBV AND ICC PROGNOSIS

ICC is notoriously predictive of a poor prognosis, mainly due to poorly encapsulated tumors, periductal invasion, frequent lymphatic involvement, or a greater difficulty of making early diagnoses compared to HCC. Furthermore, these characteristics are more prominent in ICC patients with seronegative-HBsAg than in ICC patients with seropositive-HBsAg[21]. Recent studies also found that high preoperative CA19-9 levels in ICC or CK19 expression in some tumors resulted in a worse prognosis after surgical treatment[47-50]. Compared to HBV-associated ICC, CK19 expression and high CA19-9 levels were found to occur more frequently in ICC in the absence of HBV infection[47,48,50]. Additionally, patients with chronic HBV infection often undergo surveillance with AFP and ultrasound every 3-6 mo for the detection of early HCC, which may lead to the unexpected detection of ICC at a relatively early stage; thus, the proportion of ICC patients who undergo curative resection is significantly higher for HBV-positive than HBV-negative patients[38]. Taken together, relative to ICC without HBV infection, HBV-related ICC shows a trend toward a lower proportion of most of the aforementioned malignant properties and a higher proportion of patients who could be diagnosed early and have undergone curative resection. These results may indicate that ICC patients with HBV infection have a more favorable prognosis compared to ICC patients without HBV infection. Indeed, this hypothesis has been further supported by some recent studies [38,47,51].

POTENTIAL PATHOGENESIS OF HBV-ASSOCIATED ICC

Although epidemiological evidence based on the statistical analyses of patient samples strongly supports the causal role for HBV and HBV-related cirrhosis in the development of ICC, the pathogenesis of HBV-mediated intrahepatic cholangiocarcinogenesis remains largely unknown. To date, only very few studies have been conducted to explore the pathogenic mechanisms of HBV-related ICC.

Cancer stem cells

Recent evidence suggests that some cancers may originate from cancer stem cells, which may derive from the carcinogenesis of normal stem cells[52-55]. A hepatic progenitor cell population, also called oval cell, which gives rise to hepatocytes and cholangiocytes, has been suggested in humans, and the carcinogenesis of such hepatic progenitor cells may cause ICC[54,56]. AFP, with a molecular weight ranging from 68 to 73 kDa in dependence on carbohydrate glycoprotein, is normally produced during fetal development by the fetal hepatocytes, yolk sac cells and gastrointestinal cells[57,58]. The protein levels rapidly decrease after birth, and only trace amounts are detectable in the serum by the second year of life. However, AFP is increased in most of HBV-associated HCC patients. Hepatic progenitor cells were also shown to strongly express AFP mRNA and to produce AFP during differentiation[57,59]. Previous studies reported that HBV-associated ICC patients exhibited a higher incidence of AFP, > 20 μg/L (or > 200 μg/L), than ICC patients without HBV infection[21,26,42,60]. These results may indicate that one mechanism for the development of ICC involves the neoplastic transformation of oval cells and that the oval cell precursors retain the ability to produce AFP through the process of malignant transformation.

Similarities in the demographics of HBV-associated HCC and ICC have been observed, suggesting that common disease processes may be involved. HBV-associated ICC and HCC patients were reported to be younger and more frequently male than ICC patients without HBV infection[21,26,42], and the age and sex distribution profiles were nearly identical between HBV-associated ICC patients and HBV-associated HCC patients[21]. HBV-associated ICC or HCC occurs on average 10 years earlier than HCV-related ICC or HCC[28], and the age profiles of HBV-associated ICC and HCC and HCV-associated ICC and HCC are also similar[28]. The incidence ratio of HCC:ICC: combined hepatocellular cholangiocarcinoma was found to be consistent with the theoretic ratio of hepatocyte number to cholangiocyte number in the liver[28]. Taken together, researchers concluded that HBV-associated ICC and HCC hold common disease processes and that the two types of tumors evolve from hepatic progenitor cells. Using unique mouse models and eloquent hepatocyte fate tracing methods, Fan *et al*[61] and Sekiya and Suzuki[62] have independently demonstrated a compelling alternative to the cellular origin of ICC, namely, through the transdifferentiation and neoplastic conversion of normal hepatocytes into malignant cholangiocytes *via* a mechanism mediated in part by the overexpression of activated Notch. Of further interest, in both studies, ICCs were observed to originate from transdifferentiated hepatocytes in the central areas of the liver lobule and not in the periportal areas, the site of the hepatic stem/progenitor cell niche.

Role of chronic inflammation and cirrhosis

It is well known that most HCC arise in the context of HBV-associated cirrhosis in HBV-endemic areas, clearly suggesting that cirrhosis is the most important risk factor of HCC and is a pre-neoplastic condition per se. The strong association between cirrhosis and HCC suggests a hepatocarcinogenic process that is largely mediated by inflammation, leading to repeated cycles of cell death and regeneration that increase hepatocyte proliferation turnover. Although HBV infection and cirrhosis are generally considered to be unrelated to the mechanism of ICC carcinogenesis[63], a large amount of epidemiological data in recent strongly support that HBV infection

may play an important etiological role in ICC development. Our previous studies have also showed that HBV-associated cirrhosis and histological inflammation are significantly higher in HBV-associated ICC patients than in ICC patients without HBV infection[21,26]. These results indicate that HBV-associated HCC and HBV-associated ICC may share a common disease process for carcinogenesis, through a similar long-term inflammatory carcinogenic process.

Role of HBx protein

The HBx protein (17 kDa) communicates with a variety of host targets and disturbs cellular functions, including cell cycle regulation, apoptosis, signaling, transcriptional regulation, and the expression of cytoskeleton, cell adhesion molecule, tumor suppressor genes, and oncogenes[64-70]. HBx plays a crucial role in HCC development[71-75], up-regulating the expression of such proto-oncogenes as c-myc[76] and c-Jun[77], such transcription factors as nuclear factor kappa B (NF-κB)[78], AP-1[79], AP-2[80], and cyclic adenosine monophosphate response element binding protein[81], and such other viral genes as HBV enhancers in the nucleus[82]. HBx activates mitogen-activated protein kinase (MAPK)/mitogen-activated protein kinase (ERK), stress-activated protein kinase/Jun N-terminal kinase, and protein kinase C signaling pathways to regulate NF-κB and AP-1-dependent transcription[78]. The induction of NF-κB and AP-1 activity leads to the acceleration of cell cycle progression, increased proliferation, and the suppression of apoptosis. HBx can bind to the C-terminus of p53, forming a protein-protein complex and inactivating many functions of p53, including apoptosis. HBx also inhibits the repair of damaged liver cell DNA by interacting with p53 or by binding to the damaged DNA-binding protein, which is implicated in DNA repair and cell cycle regulation, leading to the accumulation of DNA mutations and cancer[83,84]. Similar carcinogenic effects of HBx are expected to occur in ICC. Wang *et al*[34] reported that the *HBx* gene had a high positive rate in cancerous tissues and surrounding liver tissue. Zhou *et al*[37] found that the HBx protein was frequently expressed in the surrounding liver tissues of HBV-associated ICC and that patients with HBx expression had a significantly higher prevalence of elevated serum AFP. Collectively, these results indicate that HBx may also play an important role in the pathogenesis of ICC. Zhou *et al*[37] also found that 33.3% of HBV-relative ICCs exhibited p53 protein expression, though p53 protein expression was not correlated with HBx expression. Given this result, Zhou *et al*[37] concluded that p53 abnormality may not play a significant role in HBx-mediated oncogenicity during ICC carcinogenesis. Performing *in vivo* research in models, Liu *et al*[84] found that the pSmad3L oncogenic pathway was activated in HBx and HCP (hepatitis C virus core)-induced ICC and involved the phosphorylation of p38 by MAPK and p44/42 ERK1/2, indicating the association with the transforming growth factor beta 1 (TGF-β1) signaling pathway in ICC. The knockdown of TGF-β1 by *in vivo* morpholino injections markedly reduced bile duct proliferation, fibrosis, and ICC. These results reveal that TGF-β1 plays an important role in HBx- and HCP-induced ICC development.

CONCLUSION

A large amount of epidemic data strongly support that HBV infection plays an important etiological role in ICC development, particularly in HBV-endemic areas. HBV-associated ICC holds many clinicopathological similarities with HBV-associated HCC, and HBV-associated ICC patients may have a better prognosis than ICC patients without HBV infection. HBV-associated ICC and HBV-associated HCC appear to share a common disease process for carcinogenesis, a similar long-term inflammatory carcinogenic process, and both possibly arise from hepatic progenitor cells.

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