Hepatitis B and C viruses in the development of hepatocellular carcinoma

Ming-Whei Yu, Chien-Jen Chen*

Institute of Epidemiology, College of Public Health, National Taiwan University, No. 1 Jen-Ai Rd. Sec. 1, Taipei 10018, Taiwan

Accepted 9 November 1993

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* Corresponding author. Tel. 886 2 393 4392; Fax. 886 2 351 1955.

1. Introduction

The hypothesis that a virus may contribute to the development of cancer has been proposed since the 1910s [1]. Certain DNA- or RNA-containing viruses have been recognized as inducing tumors in animals [1]. Associations between human viruses and the induction of certain cancers have also been established on the basis of epidemiological, clinical, and molecular biological studies. Although there is substantial evidence suggesting the importance of certain subtypes of human papilloma virus in the development of cervical cancer, Epstein-Barr virus in the development of Burkitt's lymphoma and nasopharyngeal carcinoma, as well as HTLV-I in the pathogenesis of adult T-cell leukemia [2], among the human oncogenic viruses the link between hepatitis B virus (HBV) and hepatocellular carcinoma (HCC) is the strongest ever demonstrated.

HCC is one of the most common cancers in the world. However, there exists a striking geographic variation in its incidence throughout the world [3]. The highest incidence areas cluster in south-eastern Asia and sub-Saharan Africa. HBV has been well-documented as the major etiological factor of HCC in these areas where it accounts for 50–90% of the liver cancer cases.

A large proportion of HCC cases in developed countries have long been noted to have no detectable serological markers of HBV infection [4]. This implies that other risk factors may contribute to the pathogenesis of HCC. There were several convincing case reports that described the development of HCC following non-A, non-B transfusion-associated hepatitis [5–8]. However, the association between non-A, non-B post-transfusion hepatitis and HCC could be investigated only recently after the hepatitis C virus (HCV) genome had been cloned and specific assays for circulating anti-HCV were developed [9]. HCV has been shown to be the main causative agent in parenterally transmitted non-A, non-B hepatitis [10]. The relative importance of HCV in the etiology of HCC has been particularly emphasized in Japan, Spain and Italy.

Although a vast majority of HCC cases has been attributed to persistent viral hepatitis infections, numerous data obtained from various epidemiological study designs suggest that other factors may also contribute to the development of HCC. A moderate excess risk of HCC has been associated with cigarette smoking and habitual alcohol drinking [11–15]. Recent nested case-control studies also suggested that aflatoxin exposure and elevated serum level of endogenous testosterone may play a significant role in the development of HCC [16,17].

The evolution of HCC is a process of multiple stages with the involvement of multiple factors. HCC usually develops after several decades of persistent infection with HBV or HCV. The relatively long latency period

between the initial viral infection and HCC development, as well as the fact that only a small fraction of infected individuals eventually develop HCC, implies that the virus itself may not be a sufficient cause for the development of HCC. Other factors may be required in the virus-related hepatocarcinogenesis. Researches aimed at the investigations of the interactions of HBV or HCV with other risk factors may provide further insight into the multifactorial etiology of HCC.

Viruses can contribute to the development of human cancers by a variety of mechanisms including stimulation of cell proliferation through viral oncogenes or causing cellular injury, insertional mutation, and immunosuppression [2]. A number of molecular biological and animal studies have provided important etiological insight into the mechanism of HBV-related hepatocarcinogenesis. However, many uncertainties about the precise mechanism in humans still exist. They continue to challenge scientists interested in the prevention, biology and therapy of HCC. The understanding of the role of HCV in the pathogenesis of HCC also remains primitive. The objective of this review is to address the sources of evidence concerning the roles of HBV and HCV in the etiology of HCC, with emphasis on the interactions between the viral infection and other HCC risk factors which have not been sufficiently evaluated.

2. Descriptive epidemiology of HCC

2.1. Demographic characteristics

In populations at an HCC risk ranging from the lowest to the highest, the incidence of HCC increases with age. However, the age-specific incidence of HCC differs appreciably in different geographical areas, especially between populations at very high and low risk [18]. In western countries, HCC is primarily a disease of the elderly, with most HCC patients diagnosed at ages 60 and older [19]. However, the ages shift markedly to the younger age groups in areas of high incidence. In much of Asia, the average age of those affected with HCC is around 50, and around 40 in Africa. In certain high risk areas of Africa, the age curve of HCC incidence peaks at 25 years of age and then shows a plateau with only a slight decline after 50 years of age [18]. Such an age curve suggests the existence of certain risk factors of HCC which may have a strong effect in childhood.

Men are more frequently affected with HCC than women. The sex discrepancy in HCC incidence is evident throughout the world [3,19,20]. In Taiwan, HCC is two- to three-times more frequent in men than in women, despite their similarity in HBsAg carrier rate [20]. Differences in sex hormone and life-style habits such as alcohol drinking and cigarette smoking have been implicated in the gender discrepancy [11,17].

2.2. Geographical variation

HCC is rare or uncommon in most parts of the world. However, this cancer is very common throughout sub-Saharan Africa and in many parts of the Far East [3,21]. Although the international geographical variation in the incidence of HCC has been correlated to the prevalence of HBsAg carrier status [3,21], these ecological correlation studies may be confounded by the aflatoxin exposure because the ingestion of foods contaminated with a relatively high level of aflatoxins is common in populations living in high risk areas. The variation in HCC incidence within countries may also be partly explained by variation in the prevalence of HBsAg carrier status [3,22]. However, HBsAg carrier rate may not readily explain all the intra- or international variation of HCC incidence. An ecological correlation study in Taiwan reported the mortality rate of liver cancer is significantly higher in the mountainous aborigines, Penghu Islets, and in endemic areas of blackfoot disease, a peripheral vascular disease induced by chronic arsenic exposure [20]. As the HBsAg carrier rate was only slightly higher in these areas than in the general population in Taiwan, other factors unique in these areas have been considered to play at least a partial role in the development of HCC.

2.3. Migrant studies

The extraordinarily high incidence of HCC among Chinese throughout the world is one of its most significant characteristics. In Singapore, the age-adjusted rate is higher in Chinese than in Indians and Malays. In San Francisco and Los Angeles, Chinese also have a significantly higher age-adjusted rate of liver cancer than blacks, whites and Japanese [20]. As most chronic HBV carriers among Chinese are infected with this virus during early childhood through vertical transmission, the consistently high HCC incidence among Chinese migrants supports the role of HBV in the etiology of HCC.

2.4. Time trends

An analysis of 37 populations from 18 countries with cancer registry systems showed a significant increase in HCC incidence among 17 (46%) of the populations studied for men and among 10 (27%) for women between 1956 and 1972 [23]. Improvement in the accuracy of diagnosis and registration might account for this increase during this period. In countries characterized by high prevalence of HBsAg carrier status such as Singapore, Hong Kong and Taiwan, the secular trend of HCC in recent years has shown different patterns. The HCC incidence remained relatively constant or slightly declined among both men and women between 1968 and 1982 in Singapore and Hong Kong [24,25]. In Taiwan, the age-adjusted mortality of liver cancer has increased among men during the same period, but remained stable

among women [20]. As the HBsAg carrier rate is relatively stable for both men and women in Taiwan, the rising HCC trend for men suggests men in Taiwan may have either increasingly been exposed to environmental carcinogens other than chronic HBV infection or changed their life-style habits predisposing to the development of HCC. The most striking increase in the HCC incidence in recent decades was reported from a study in Japan in which a more than two-fold increase in HCC incidence was observed among men [26]. Non-A, non-B post-transfusional hepatitis has been considered as a possible cause of the increase in HCC incidence.

3. HBV and HCC

3.1. Epidemiological studies

Epidemiological studies have provided strong evidence for the association between chronic HBV infection and HCC. In the case-control studies, summarized in Table 1, the relative risk of developing HCC associated with HBsAg carrier status ranged from 5 to 20. Our three previous case-control studies in Taiwan also reported a higher HCC risk for carriers of both HBsAg and HBeAg than for carriers of HBsAg alone [11,31,32].

The causal relationship between chronic HBV infection and the development of HCC has been addressed in six cohort studies (Table 2). Two large cohort studies in Taiwan examined the association of HBsAg carrier status with HCC by comparing the HCC incidence among HBsAg carriers with a non-carrier control population [15,39]. In four cohort studies of HBsAg positive blood donors in Japan, and England and Wales, the observed number of deaths from HCC was compared with the expected number derived from the age, sex, and calendar time-specific mortality from this cancer in the general population [40–43]. The estimates of relative risk for HBsAg carrier status varied from 6 to 100. The highest relative risk was reported from a prospective study of government employees in Taiwan [39]. This relative risk is much higher than those observed in our community-based cohort study in Taiwan [15] and several case-control studies in Hong Kong [29], mainland China [30] and Taiwan [11,31,32]. The explanation for this difference is not clear. Perhaps HCC risk factors other than chronic HBV infection are less common among government employees in Taiwan so the relative importance of HBsAg carrier status is higher.

The fraction of HCC attributable to HBsAg carrier status varies from 50% to more than 90% in high risk areas, 7–50% in intermediate risk areas, and less than 10% in low risk areas. The large variation in the attributable proportion varies strikingly from country to country and reflects the relative importance of chronic HBV infection in the etiology of HCC.

Table 1
Summary of case-control studies on the relation of HBsAg carrier status to risk of HCC

Author(s), published year [reference no.]	Area	Study subjects	HBsAg assay	Additional variables adjusted for	Relative risk (95% confidence interval)	Population attributable risk percentage
<i>High risk areas</i>						
Prince et al., 1975 [27]	Senegal, Africa	165 HCC cases 328 hospital controls	RIA	None	12.4 ^a (7.8-19.7) ^b	56.3 ^c
Lingao et al., 1981 [28]	Philippines	104 HCC cases 84 controls (35 hospital personnel and the rest were not connected with hospital)	RPHA	None	10.8 ^a (5.3-20.9) ^b	63.9 ^c
Lam et al., 1982 [29]	Hong Kong	107 HCC cases 107 age-matched hospital controls	RIA	None	21.3 (10.1-45.9)	78.5 ^c
Yeh et al., 1985 [30]	China	50 HCC cases 50 age-sex-matched hospital controls	RIA	None	17.0 (4.3-99.4)	77.9 ^c
Lu et al., 1988 [31]	Taiwan	131 HCC cases 207 hospital controls	RIA	Age, sex	21.5 (12.2-37.9) ^b	81.0 ^c
Chen et al., 1991 [11]	Taiwan	200 HCC cases 200 age-sex-ethnicity-residence- matched community controls	RIA	Cigarette smoking, alcohol drinking, family HCC history	HBsAg(+)/HBeAg (-): 16.7 ^d HBsAg(+)/HBeAg(+): 54.5 ^d	78.9
Yu et al., 1991 [32]	Taiwan	127 HCC cases 127 age-sex-ethnicity-residence- matched community controls	RIA	Anti-HCV status, cigarette smoking, alcohol drinking, peanut consumption	HBsAg(+)/HBeAg(-): 24.8 (7.6-80.9) HBsAg(+)/HBeAg(+): 33.5 (6.6-170.9) 15.2 (2.3-102.8)	69 for HBsAg alone; 6 for both anti-HCV and HBsAg 48.0 ^c

Srivatanakul et al., 1991 [33]	Thailand	65 HCC cases 65 age-sex matched hospital controls	EIA	Alcohol drinking, consumption of shrimp paste, powdered peanut, fresh vegetables and betel nut chewing	Men 21.4 Women 27.1
<i>Intermediate risk areas</i> Tsukuma et al., 1990 [34]	Japan	229 HCC cases 266 hospital controls	RPHA	Age, sex, blood transfusion, cigarette smoking, alcohol drinking, family HCC history	14.3 (5.7-36.3)
Trichopoulos et al., 1987 [35]	Greece	194 HCC cases 456 hospital controls	RIA	None	47.9 ^c
Mayans et al., 1990 [36]	Spain	96 HCC cases 190 age-sex-matched hospital controls	RIA	Age, sex	7.6 ^c
<i>Low risk areas</i> Yarrish et al., 1980 [37]	United States	34 HCC cases 38 colon cancer patients 45 lung cancer patients 56 blood donors as controls	RIA	None	Not available
Austin et al., 1986 [38]	United States	67 HCC cases 63 hospital controls	RIA	None	Not available

RIA, radioimmunoassay; RPHA, reverse passive hemagglutination assay; EIA, enzyme immunoassay.

^aRelative risk calculated from data in the paper.

^bConfidence interval calculated from data in the paper using Woolf's method.

^cPopulation attributable risk percentage calculated from data in the paper.

^dConfidence interval not available.

Infinity, HBsAg positive rate was significantly higher in cases than in controls (14.7% vs. 0%)
Infinity (3.8-infinity), 18% of HCC cases were HBsAg-positive, all controls were HBsAg-negative

Table 2
Summary of cohort studies on the relation of HBsAg carrier status to risk of HCC

Author(s), published year [reference no.]	Area	Study subjects	HBsAg assay	Duration (years) of follow-up	Relative risk (95% confidence interval)	Population attributable risk percentage
<i>High risk areas</i> Beasley et al., 1988 [39]	Taiwan	161 HCC incident cases 22 707 male government employees (81.6% 40-59 yr)	RIA	8.9 (average)	98.4 (50.2-193.0)	93.7 ^a
Chen et al., 1993 [15]	Taiwan	39 HCC incident cases 9688 male adults (58.5% > 50 yr)	HBsAg status of HCC cases tested by RPHA and RIA, other non-HCC cases tested by RPHA	5.2 (average)	17.0 (8.7-33.1)	67.4 ^a
<i>Intermediate risk areas</i> Tokudome et al., 1987 [40]	Japan	4 liver cancer deaths 3769 female HBsAg-positive blood donors (72.7% < 40 yr)	Electrosyneresis and RPHA	5.1 (average)	5.6 ^b (1.1-14.3) ^c	6.5
Tokudome et al., 1988 [41]	Japan	15 liver cancer deaths 2595 male HBsAg-positive blood donors (81.8% < 40 yr)	Electrosyneresis and RPHA	5.9 (average)	7.3 ^b (4.1-12.0) ^c	11.1 ^d
Oshima et al., 1984 [42]	Japan	20 liver cancer cases 8646 male HBsAg-positive blood donors (91.4% < 40 yr)	Electrosyneresis	6.2 (average)	6.6 ^b (4.0-10.2) ^c	10.1 ^d
<i>Low risk areas</i> Hall et al., 1985 [43]	England and Wales	5 liver cancer deaths in men No deaths from liver cancer in women 2880 male and 1054 female HBsAg- positive blood donors (median age: 25 yr in men and 20 yr in women)	Not stated	13	42.0 ^b (13.6-97.9) in men	3.9 ^e

RIA, radioimmunoassay; RPHA, reverse passive hemagglutination assay.

^aPopulation attributable risk percentage calculated from data in the paper.

^bStandardized mortality ratio.

^cConfidence interval of standardized mortality ratio calculated from data in the paper using exact Poisson limits.

^dPopulation attributable risk percentage calculated from data in the paper, based on a 2% of HBsAg carrier rate in Japan.

^ePopulation attributable risk percentage calculated from data in the paper, based on a 0.1% of HBsAg carrier rate in general population in England.

3.2. Experimental studies

3.2.1. Animal models. The development of HBV-related HCC has been studied in many animal models. HBV belongs to the family of hepadnavirus which shows great genomic and biologic homologies but limited cross-infectivity [44]. In addition to HBV, three hepadnaviruses including woodchuck hepatitis virus (WHV) [45,46], ground squirrel hepatitis virus (GSHV) [47], and duck hepatitis B virus (DHBV) [48–50], have been found to cause persistent infection and a variety of liver diseases similar to HBV including acute and chronic hepatitis, characterized by hepatocyte necrosis, inflammatory reaction as well as liver regeneration, and HCC in infected animals. Animal studies with these hepadnaviruses have provided further insight into the relationship of chronic HBV infection to the development of HCC.

The original model, developed by Summers et al. in 1978, showed that persistent WHV infection was associated with a high incidence of HCC in captive woodchucks [45]. Popper et al. investigated the evolution of experimental WHV infection of woodchucks [46]. The most striking finding was that HCC developed within 3 years after infection in 100% (8/8) of experimentally-infected chronic carriers of WHV surface antigen (6 infected at birth, 2 infected in adulthood) in the absence of an external co-carcinogen under well-controlled conditions. There was no HCC observed in laboratory animals having a past WHV infection without becoming carriers or in uninfected control animals.

In the study of GSHV-induced HCC, colonies of GSHV-infected and uninfected captive ground squirrels were followed for a five-year period [47]. HCC appeared in 2 of 28 GSHV carrier animals and 1 of 23 squirrels with past GSHV infection, while no HCC were observed in animals without serologic evidence of current carrier status or past GSHV infection. The relatively low incidence of HCC in carrier ground squirrels was in great contrast to the high HCC incidence observed in woodchucks chronically infected with WHV. This discrepancy was suspected to be due to viral and/or host susceptibility factors. A recent study compared the oncogenic potential of GSHV and WHV in woodchucks [51]. Despite the similarity in the replication of WHV and GSHV in experimentally-infected woodchucks, HCC developed in GSHV carriers about 18 months later than in WHV carriers. The frequency of HCC was also lower in GSHV-infected woodchucks than in WHV infected woodchucks. This observation implied that the discrepancy in the oncogenic potential between the two hepadnaviruses may depend on specific determinants in viral genomes.

Although the mammalian hepadnaviruses such as WHV and GSHV have been associated with the development of HCC, the association of DHBV with HCC in ducks is less clear. HCC was reported in DHBV-infected ducks from a region of China where HCC is

common in both ducks and humans [48]. However, HCC has not been observed in persistently DHBV-infected domestic ducks in the United States. There was no evidence for the induction of HCC in experiments with congenitally DHBV-infected ducks [49,50].

Besides the animal studies on hepadnavirus, evidence for the link between HBV and human HCC comes from experiments with transgenic mice carrying different HBV genes. Chisari et al. constructed transgenic mice that overexpress the HBV large envelope polypeptide [52]. The dysregulation of the HBV protein causes its accumulation in hepatocytes and then severe, prolonged cellular injury that triggers a cascade of events including inflammation, regenerative hyperplasia, chromosomal aberrations, and eventually the development of HCC. Dunsford et al. conducted a study in which 59 transgenic mice that overproduced the HBV large envelope polypeptide and 9 age-sex-matched non-transgenic littermate controls were followed for evidence of HCC throughout their 24-month life span [53]. All the transgenic mice developed HCC at ages between 1 and 2 years, but no histopathological changes were observed in the non-transgenic controls over the same period of observation.

HBV may predispose individuals to liver carcinogenesis directly by its viral gene products. Kim et al. investigated the role of *X* gene of HBV (HBx), which has been demonstrated to encode a protein acting as a transcriptional transactivator, in the pathogenesis of HCC in a HBx gene transgenic mouse model [54]. The high expression of HBx gene in the liver provoked progressive morphological changes beginning with multifoci of altered hepatocytes, followed by the appearance of benign adenomas, and proceeded to the development of HCC. HCC developed in most of the transgenic mice but in a relatively low percentage of controls.

A recent study also reported that a non-malignant fetal mouse hepatocyte cell line transfected with HBV DNA displayed malignant growth characteristics in soft agar and caused the formation of HCC upon inoculation in nude mice [55]. This result suggested that HBV genome has oncogenic potential.

In summary, most of the animal studies mentioned above have provided evidence for a role of HBV in the development of HCC, except for the experiments with DHBV-infected ducks. Although these animal models have improved our understanding of the hepatocarcinogenic mechanisms induced by HBV, the earlier onset of HCC and the nearly 100% risk for the development of HCC in certain experimental animals are different from those observed in most human hepatomas. No external co-factors required in the pathogenesis of HCC in these animal models might also be different from HBV-induced hepatocarcinogenesis in humans which usually needs a relatively long incubation period of several decades and multifactorial involvement.

3.2.2. Molecular studies. The integration of HBV into

cellular DNA has been detected in approximately 90% of liver tissues from HBsAg-positive HCC patients and many HCC cell lines [56–68]. The fact that the integrated sequences of HBV genome are also present in liver tissues of chronic HBV carriers and patients with HBV-related chronic liver diseases [57,60,61,68] suggests the viral integration may occur during the long-lasting period of chronic HBV infection.

Southern blot analyses of HBV DNA in HCC have shown that these tumors are clonal with respect to the integration pattern of HBV DNA [56–59]. This implied that the viral integration may proceed or accompany the transforming event in HCC induction. The possible roles of the integration of HBV into host genome at the initial stage of hepatocarcinogenesis have been intensively investigated.

Integration of HBV DNA into cellular genome may cause chromosomal alterations including deletions [69–71], translocations [72–74] and duplications [72,75–77]. The ability of HBV to induce chromosomal aberrations is consistent with its potential role as a tumor initiator. The loss of chromosome has been linked to the loss of functional tumor suppressor genes in many human cancers [78]. Deletion in chromosome 11p that contains the Wilm's tumor locus has been reported to be associated with a HBV integration site in HCC [69–71]. Although the deletion of chromosome 17p near the *p53* gene was frequently observed in HBV-integrated HCC in an area where high exposure to aflatoxins has been suspected of causing a high frequency of point mutations on *p53* locus, especially in the 249th codon, of HCC tissues [79], there was no direct evidence that the 17p deletions were associated with HBV integrations [69,80]. Chromosomal translocation may also be involved in the development of HCC. However, unlike the specific translocations observed in a variety of hematopoietic neoplasms [81], no common translocation was identified in HCC. Translocations between chromosomes 5:9, X:17 [72], 17:18 [73], and 17:7 [74] at the HBV integration site have been reported.

HBV may also act as an insertional mutagen by the integration of viral DNA into host genome and activating cellular proto-oncogenes in the flanking host DNA. The insertional activations of *N-myc* or *c-myc* genes by WHV DNA integration have been reported in one third of HCCs in woodchucks [82]. These findings implied that WHV may contribute to the genesis of liver tumor, like non-acute retroviruses, through insertion of viral enhancer and disruption of normal proto-oncogene expression. However, this mechanism may not be operative in most HBV-related HCCs. In humans, non-specific integration of HBV DNA into multiple cellular DNA sites in liver tumor tissues was frequently observed [59,61,62,65,66,68]. A study investigating a total of 14 paired human HCC and adjacent non-tumorous tissues found no relationship between the acti-

vation of *ras* and *c-myc* oncogenes in HCC and the expression of *HBV* gene or the presence of viral DNA/RNA at the cellular level [83]. So far there have been very few documented HCC cases with HBV integration site within or near the cellular genes crucial for growth control and differentiation [84–88].

An alternative way for the integration of HBV DNA into host genome to be involved in the evolution of HCC is through the transactivation of cellular genes by gene products encoded by the integrated HBV DNA. The X protein translated from the X open reading frame of HBV genome has been known as a non-specific activator of transcription [89–98]. Most integrated HBV DNAs preserved the major part of its X gene [76,99]. A large proportion of HBV carriers with HCC have been reported to be positive for the X antigen in their tumor cells [100]. Although the loss of 3' end region of X gene due to integration was frequently observed, many such truncated products retain transactivation activity [93]. Activations of a variety of cellular and viral genes by X protein *in vitro* have also been demonstrated [89–98]. A recent study also suggested that the X protein was a novel serine/threonine protein kinase [101]. As certain retroviral oncogenes known to encode protein kinase have been shown to induce transformation of cells in culture and oncogenesis *in vivo* [102,103], the X gene may have the potential to alter cell growth and differentiation by catalyzing phosphorylation of cellular factors involved in the regulation of transcription. A fraction of HCC patients may not contain a functional X gene [100]. The *HBV* gene product translated from truncated pre S2/S sequences of integrated HBV DNAs has also been reported to have a transcriptional transactivation activity [104,105]. However, further studies are required to determine the percentage of HCCs which express a truncated pre S2/S protein with a transcriptional transactivating activity and how important is the role of this protein in hepatocarcinogenesis.

3.2.3. Possible mechanisms of HBV-related hepatocarcinogenesis. Table 3 summarizes current views of the mechanisms through which HBV infection may cause the development of HCC. HBV may function as a tumor initiator through a non-specific mechanism to cause cellular DNA rearrangements [72,75,77] or to augment expressions of cellular growth genes by the action of X protein [89–98]. As chromosomal aberration is frequently observed in liver tumor tissues, the unstable nature of HBV integrated cellular DNA may be a common mechanism for the HBV-related hepatocarcinogenesis. However, the rearrangement of cellular DNA at HBV integration sites occurs not only in HCCs but also in HBV chronically-infected liver tissues [76]. The exact role played by the rearrangement of cellular gene in the development of HCC is unclear. Transactivation of cellular genes by the products of integrated HBV pre S2/S or X gene has been proposed to explain the connec-

Table 3
Possible mechanisms of HBV-related hepatocarcinogenesis

Mechanism ^a	Comment
Integration of HBV DNA into host hepatocyte may cause an unstable structure of cellular DNA and therefore chromosomal aberrations [72,75,77]	Chromosomal aberrations are frequently observed in human HCCs. Rearrangement of cellular DNA at HBV DNA integration sites is non-specific for HCCs [76]. Specific chromosomal aberrations associated with all HBV-related HCCs have not been yet found.
a. Induce chromosomal deletion del 11p [70,71]	
b. Induce chromosomal translocation t(17, 18) [73] t(17, 7) [74] t(5, 9) [72] t(X, 17) [72]	
HBV X [89–98] and pre S2/S (104, 105) gene products have a transcriptional trans-activating function. Integration of HBV X or pre S2/S gene sequence may cause inappropriate expressions of cellular genes.	No evidence supports this hypothesis in human.
Site-specific integration of HBV DNA into host genome may directly activate cellular oncogenes and/or inactivate tumor suppressor genes.	
a. HBV DNA integrates within or closely to cellular genes critical for growth control Retinoic acid receptor gene [84, 85] cyclin A gene [86] p53 gene [87]	Site-specific integration of HBV DNA in human HCCs is very infrequent and may not readily explain the majority of HBV-associated HCCs.
b. Cause overexpression of cellular oncogenes hst-1 gene [88] C-myc gene (woodchuck model) [107,108] N-myc gene (woodchuck model) [82]	Overexpression of known oncogenes has not been observed as a common phenomenon of HBV-related HCCs in human [83, 109].
Persistent HBV infection can cause prolonged hepatocellular injury characterized by chronic phasic necroinflammation and then induce regenerative hyperplasia of hepatocyte and eventually the malignant transformation [46,52,53,110].	Chronic hepatitis and liver cirrhosis are common sequence of chronic HBV infection. Over 85% of HBV-related HCCs have cirrhosis [111]. An elevated risk of HCC has also been observed in alcoholics and patients with hemochromatosis or α_1 -antitrypsin deficiency.

^aReference numbers shown in square brackets.

tion between non-specific integration of HBV DNA and HCC development [89–98,104,105]. The findings from the HBx gene transgenic mouse model [54] and several in vitro studies [89–98] suggest that the expression of the HBV X gene in hepatocytes may contribute, at least partly, to the development of HCC. However, which cellular genes that are involved in the pathogenesis of human HCC may be activated by X gene remain to be determined. No evidence for a specific cellular integration site of HBV DNA has been reported to date [106]. In addition, there were only a limited number of documented HCC cases reported to have HBV insertions within or next to cellular genes associated with cell growth control and differentiation program [84–88]. This indicated that the insertional mutation accounts for a tiny fraction of human HCCs.

HCC usually develops after 20–30 years of persistent HBV infection accompanied by the signs of hepatocyte necrosis, inflammation, and regenerative hyperplasia in chronic HBV carriers. Chronic hepatitis and liver cirrhosis have long been suggested to be important endogenous factors in the development of HCC. This implies that the main mechanism for HBV to predispose individuals to HCC may be through the hepatic injury and non-specific regenerative proliferation, rather than a direct genetic event. The continuous hepatocyte

regeneration may allow an accumulation of multiple mutational events and provide a growth stimulation to a rare cell that already has a genetic change due to random mutation or the integration of HBV genome. The fact that there is a much higher risk for developing HCC among chronic HBV carriers than individuals with past HBV infection is consistent with this explanation and argues against the importance of the hit-and-run mechanism by insertional mutation in the HBV-induced hepatocarcinogenesis. The WHV animal model has emphasized the role of necroinflammation in the development of HCC [46,110]. The findings from the HBV envelope transgenic mouse model provide important evidence for the molecular mechanism of hepatic injury responsible for HCC induction [52,53]. Indeed, no changes in known oncogenes and tumor suppressor genes were observed during hepatocarcinogenesis in the transgenic mouse model [112]. Although the pathogenesis of hepatic injury in HBV-related liver diseases may be mediated by both virological factors and host immune system rather than by the dysregulation of the HBV envelop protein [113], the HBV transgenic mouse may provide a good model for the investigation of the association between the chronic hepatic damage and continuous liver regeneration and the development of HCC.

4. HCV and HCC

4.1. Non-A, non-B hepatitis and HCC

The application of sensitive immunological assay in the 1970s for testing and differentiating infections with hepatitis A and B viruses led to the identification of a distinct type of infectious hepatitis, namely non-A, non-B hepatitis, which was negative on serological markers of known hepatotropic viruses such as hepatitis A virus, hepatitis B virus, cytomegalovirus, and Epstein-Barr virus. Non-A, non-B hepatitis has been shown to be the most common serious consequence of blood transfusion; it accounts for approximately 85–95% of post-transfusion hepatitis cases. It has been reported that 7–10% of transfused patients were affected with non-A, non-B hepatitis [114].

The link between non-A, non-B post-transfusion hepatitis and HCC was proposed according to clinical observations. A long-term follow-up study indicated that a large proportion of cases of acute non-A, non-B transfusion-associated hepatitis developed chronic hepatitis defined by an elevated level of alanine aminotransferase (ALT) for more than 6 months [115]. These patients may have a persistently abnormal liver function manifested at least by an increased ALT level for several years or go on to develop an asymptomatic carrier state. A substantial fraction of these patients has been reported to progress to liver cirrhosis [114,115]. Several cases who developed HCC subsequent to chronic post-transfusion hepatitis have also been reported [5–8].

In addition to clinical observations, the evidence for the association between non-A, non-B hepatitis and HCC was from pathological studies. Liver cell dysplasia has been observed in 42.5% of the liver biopsy and surgical resection specimens from patients with various forms of non-A, non-B hepatitis including acute hepatitis, chronic hepatitis and cirrhosis [116]. This finding suggests that liver cell dysplasia and HCC may be the sequelae of non-A, non-B hepatitis.

The importance of non-A, non-B transfusion-associated hepatitis in the development of HCC was emphasized by the studies on the recent increasing trend of HCC incidence in Japan [26,117]. Although the incidence of HCC for men in Japan increased steadily between 1958 and 1983, the number of HCC cases positive for HBsAg remained almost unchanged during this period. The increasing HCC incidence has been hypothesized to be associated with non-A, non-B hepatitis on the basis of the fact that an increasing number of patients with cirrhosis and HCC who had a past history of blood transfusion but no serological markers of HBV were observed by many Japanese physicians [26]. The increased proportion of HCC cases unrelated to HBV has been confirmed by a later study on the analyses of HBV DNA in livers of HCC cases [117].

Although non-A, non-B post-transfusion hepatitis

has long been suspected of playing a role in the development of HCC, the association of the non-A, non-B hepatitis with HCC could be investigated only recently after the isolation of a blood-borne non-A, non-B hepatitis viral genome, designated hepatitis C virus (HCV), by using a recombinant complementary DNA approach. A specific assay for circulating HCV antibody has been developed [9]. Using this immunoassay anti-HCV has been detected in more than 80% of patients with chronic post-transfusion non-A, non-B hepatitis from Italy and Japan [10,118,119]. In the United States, anti-HCV has also been associated with most community-acquired non-A, non-B hepatitis with no identifiable source of parenteral exposure to HCV [10].

4.2. Anti-HCV in patients with chronic liver diseases or HCC

The prevalence of anti-HCV in patients with various chronic liver diseases has been intensively investigated (Table 4). Anti-HCV was detected in about 1% of blood donors [111,121,124,129–131]. In patients with chronic liver diseases or HCC, the percentages of anti-HCV positivity were 4–58% in HBsAg-positive cases and 43–90% in HBsAg-negative cases. The anti-HCV prevalence was consistently higher in HBsAg-negative than in HBsAg-positive patients with chronic liver diseases or HCC. The most striking discrepancy in the prevalence between HBsAg-positive and HBsAg-negative cases was observed in Japan. A high frequency of anti-HCV positivity was also observed in patients with alcoholic liver diseases. Among HCC cases, the HCC patients with cirrhosis had a higher prevalence of anti-HCV than those without coexisting cirrhosis.

The high prevalence of anti-HCV in patients with chronic liver diseases or HCC, particularly in the HBsAg-negative patients, in contrast to the relatively low anti-HCV prevalence in blood donors in different areas of different HCC incidence, suggests the HCV infection may play a role in the development of HCC throughout the world. However, these case-series studies can not be used to estimate a valid relative risk associated with HCC development for anti-HCV positivity due to the lack of appropriate comparison group.

4.3. Epidemiological studies on the association of HCV with HCC

Several case-control studies have provided information on the relation between HCV infection and HCC. In most of the case-control studies as summarized in Table 5, a significant association between anti-HCV positivity and HCC was observed with a relative risk ranging 6–62. Only one study conducted in Thailand failed to show an increased risk of developing HCC among individuals positive for anti-HCV. However, the prevalence of anti-HCV among HCC cases (6.3%) in this

Table 4
Frequency of antibody to hepatitis C virus in patients with HCC and chronic liver diseases

Author(s), published year [reference no.]	Area	Study subjects	Anti-HCV assay	Positive rate of anti-HCV
<i>High risk areas</i>				
Chen et al., 1990 [111]	Taiwan	78 HBsAg-positive chronic hepatitis cases 43 HBsAg-negative chronic hepatitis cases 31 HBsAg-positive cirrhosis cases 30 HBsAg-negative cirrhosis cases 42 HBsAg-positive HCC cases 24 HBsAg-negative HCC cases 420 blood donors	RIA	HBsAg-positive chronic hepatitis: 7.7% HBsAg-negative chronic hepatitis: 65.0% HBsAg-positive cirrhosis: 10.0% HBsAg-negative cirrhosis: 43.0% HBsAg-positive HCC: 17.0% HBsAg-negative HCC: 63.0% Blood donors: 0.95%
<i>Intermediate risk areas</i>				
Bruix et al., 1989 [120]	Spain	96 HCC cases 106 liver cirrhosis cases 177 patients admitted for various surgical procedures unrelated to liver disease as controls	EIA	HCC: 75% HCC without cirrhosis: 0% HBsAg-positive HCC with cirrhosis: 55.5% HCC with alcoholic cirrhosis: 76.6% HCC with cryptogenic cirrhosis: 81.4% Liver cirrhosis: 55.6% Controls: 7.3%
Vargas et al., 1990 [121]	Spain	81 HCC cases 33 alcoholic liver cirrhosis cases 2915 blood donors (HBsAg status not stated)	EIA	HCC: 54% Alcoholic liver cirrhosis: 24% Blood donors: 1%
Colombo et al., 1989 [122]	Italy	41 HBsAg-positive HCC cases 91 HBsAg-negative HCC cases 139 chronic liver disease cases	RIA	HBsAg-positive HCC: 54% HBsAg-negative HCC: 70% Chronic liver disease: 74%
Levrero et al., 1991 [118]	Italy	53 HBsAg-positive HCC cases 114 HBsAg-negative HCC cases 50 non-A, non-B chronic post-transfusion hepatitis cases 50 cryptogenic chronic hepatitis cases	EIA	HBsAg-positive HCC: 28.3% HBsAg-negative HCC: 71.9% Non-A, non-B chronic post-transfusion hepatitis: 94.0% Cryptogenic chronic hepatitis: 82.0%
Simonetti et al., 1989 [123]	Italy	200 HCC cases (cirrhosis = 180, without cirrhosis = 15, HBsAg-positive = 31, HBsAg- negative = 169)	EIA	Total HCC: 76% HCC with cirrhosis: 78% HCC without cirrhosis: 40% HBsAg-positive HCC: 58% HBsAg-negative HCC: 79%
Watanabe et al., 1991 [124]	Japan	23 HBsAg-positive HCC cases 102 HBsAg-negative HCC cases 56 patients with other cancers 100 blood donors negative for HBsAg and anti-HCV c100 and with normal ALT	Anti-p22 tested by Western blot, Anti-C100 tested by EIA	Anti-p22 HBsAg-positive HCC: 4.3% HBsAg-negative HCC: 83.3% Other cancers: 12.5% Blood donors: 1.0% Anti-C100 HBsAg-negative HCC: 66.7%
Nishioka et al., 1991 [125]	Japan	75 HBsAg-positive HCC cases 105 HBsAg-negative HCC cases	EIA	HBsAg-positive HCC: 14.7% HBsAg-negative HCC: 76.2%
Kiyosawa et al., 1990 [119]	Japan	50 HBsAg-positive chronic hepatitis cases 96 non-A, non-B chronic hepatitis cases 46 HBsAg-positive cirrhosis cases 81 non-A, non-B chronic hepatitis-related cirrhosis cases 29 HBsAg-positive HCC cases 54 non-A, non-B chronic hepatitis-related HCC cases	EIA	HBsAg-positive chronic hepatitis: 6% Non-A, non-B chronic hepatitis: 89.6% HBsAg-positive cirrhosis: 17.4% Non-A, non-B chronic hepatitis-related cirrhosis: 86.4% HBsAg-positive HCC: 34.5% Non-A, non-B chronic hepatitis-related HCC: 94.4%
Saito et al., 1990 [126]	Japan	51 HBsAg-positive HCC cases 202 HBsAg-negative HCC cases 148 HBsAg-negative patients with other cancers	RIA	HBsAg-positive HCC: 3.9% HBsAg-negative HCC: 66.3% Other cancers: 10.1%

Table 4 (Continued)

Author(s), published year [reference no.]	Area	Study subjects	Anti-HCV assay	Positive rate of anti-HCV
<i>Low risk areas</i>				
Nalpas et al., 1991 [127]	France	55 Patients with HCC and cirrhosis 78 alcoholic cirrhosis cases 96 non-cirrhotic alcoholic liver disease cases (HBsAg status not stated)	EIA	HCC: 58.2% Alcoholic cirrhosis: 35.9% Non-cirrhotic alcoholic liver disease: 17.7%
Hasan et al., 1990 [128]	United States	28 HBsAg-positive HCC cases 59 HBsAg-negative HCC cases (HCC patients who consumed more than 80 g alcohol daily for >10 years or those affected with hemo- chromatosis or autoimmune hepatitis or primary biliary cirrhosis or α_1 -antitrypsin deficiency were excluded) 76 cryptogenic cirrhosis cases 200 blood donors	RIA	HBsAg-positive HCC: 14% HBsAg-negative HCC: 53% Cryptogenic cirrhosis: 42% Blood donors: 0.5%

RIA, radioimmunoassay; EIA, enzyme immunoassay; ALT, alanine transferase.

study was low. The fraction of HCC attributable to HCV in terms of population attributable risk percentage varied from 9 to 29% in high HCC risk areas, 12 to 60% in intermediate risk areas, and less than 30% in the United States. Although the development of more sensitive assays to determine the presence of HCV markers may change these proportions in the future, HCV appears to be a more important cause of HCC than HBV in Japan. However, HBV is the more important etiological factor in the Far East and Africa.

The consistency of the association of anti-HCV positivity with HCC and the high relative risk among individuals positive for anti-HCV in several case-control studies suggests HCV may have a role in the etiology of HCC. However, these case-control studies examined the anti-HCV status of HCC patients and controls at the time the subjects were recruited. In other words, the anti-HCV status of HCC patients was tested after their diseases were diagnosed. The temporal relationship between HCV infection and the development of HCC cannot therefore be justified by these case-control studies. Prospective study is needed to provide unequivocal evidence that HCV infection precedes the development of HCC.

There has been only one nested case-control study using the stored sera from a community-based prospective study of liver cancer in Taiwan to investigate the association between anti-HCV status and the development of HCC to date [17]. After adjusting for other HCC risk factors such as HBsAg carrier status, habits of cigarette smoking and alcohol drinking, history of dietary consumption frequency, as well as past history of liver diseases, the relative risk of HCC associated with anti-HCV was 37.0 with a 95% CI ranging from 4.6 to 295.2. Although this study may provide the most per-

suasive evidence for the hypothesis that HCV is involved in the pathogenesis of HCC, it is unclear whether the association between anti-HCV positivity and HCC observed in Taiwan applies to other countries.

4.4. Comparison and implication of anti-HCV assays

The status of anti-HCV was determined by assays for antibodies to non-structural HCV protein c100 in most studies mentioned above. These first-generation assays have been shown to have the limitation of insensitivity and non-specificity. A fraction of chronic non-A, non-B hepatitis patients whose sera were negative for anti-HCV by the first-generation RIA were reported to have substantial levels of HCV RNA in their livers [139]. Watanabe et al. used Western blot analysis to investigate the prevalence of antibodies to HCV core protein (anti-p22) in a series of HBsAg-negative HCC patients and compared this assay with tests for detecting anti-C-100. Among 102 serum samples from HCC patients, 85 (83.3%) were positive for anti-p22, whereas 68 (66.7%) of the same 102 cases were positive for the anti-C-100 by ELISA [124]. The relatively lower sensitivity of the first-generation EIAs compared to the second-generation immunoassays, which include polypeptides derived from non-structural and structural regions of HCV genome, has also been reported recently [140].

The non-specificity of the anti-HCV immunoassays based on c100 has been demonstrated by several lines of observations [141,142]. False positivity was reported among patients with autoimmune chronic active hepatitis who had hyperglobulinemia [141]. As patients with other chronic liver diseases may also have a high serum level of globulin and most HCC patients have coexisting chronic liver diseases, similar false-positive results may be obtained in patients with HCC and thus lead to a sec-

Table 5
Summary of case-control studies on the relation of anti-HCV status to risk of HCC

Author(s), published year [reference no.]	Area	Study subjects	Anti-HCV assay	Additional variables adjusted for	Relative risk (95% confidence interval)	Population attributable risk percentage
<i>High risk areas</i>						
Kew et al., 1990 [132]	Southern Africa	380 HCC cases 152 age-sex-race-residence-matched community controls	RIA	None	61.5 ^a (8.5-445.1) ^b	28.5 ^c
Yu et al., 1991 [32]	Taiwan	127 HCC cases 127 age-sex-ethnicity-residence-matched community controls	EIA	Status of HBsAg and HBeAg, cigarette smoking, alcohol drinking, peanut consumption	23.7 (2.2-253.8)	3 for anti-HCV alone, 6 for both anti-HCV and HBsAg
Chuang et al., 1992 [133]	Taiwan	128 HCC cases 384 age-sex-matched community controls	EIA	None	Anti-HCV(+)/HBsAg(-): 27.1 (9.8-74.8) Anti-HCV(+)/HBsAg(+): 40.1 (12.6-127.6) 1.3 (0.2-8.7) Anti-HCV positive rate in HCC cases was 6.3%	16.7 ^c
Srivatanakul et al., 1991 [33]	Thailand	63 HCC cases 63 age-sex-matched hospital controls	EIA	None	—	—
<i>Intermediate risk areas</i>						
Tanaka et al., 1991 [134]	Japan	91 HCC cases 410 community controls	EIA, EIA-positive samples were retested by RIBA EIA	Age, sex, occupation, years of education	52.3 (23.9-114.3)	60.0 ^c
Kaklamani et al., 1991 [135]	Greece	185 HCC cases 432 hospital controls	EIA	Age, sex, HBsAg status	6.3 (3.7-11.0)	26.9 ^c
Zavitsanos et al., 1992 [136]	Greece	181 HCC cases 416 hospital controls	EIA	None	10.4 (4.2-26.0)	11.6 ^c
<i>Low risk areas</i>						
Yu et al., 1990 [137]	United States	51 HCC cases 128 community controls	EIA	None	10.5 (3.5-31.3)	9 for anti-HCV alone, 18 for both anti-HCV and HBV infection markers 11.4
Di Bisceglie et al., 1991 [138]	United States	99 HCC cases 98 patients with other cancers were used as controls	RIA	None	7.3 (1.8-48.2)	

RIA, radioimmunoassay; EIA, enzyme immunoassay; RIBA, recombinant immunoblot assay.

^aRelative risk calculated from data in the paper.

^bConfidence interval calculated from data in the paper using Woolf's method.

^cPopulation attributable risk percentage calculated from data in the paper.

ondary association between anti-HCV positivity and HCC. However, only a weak correlation was observed between the absorbance value in the Ortho ELISA test and serum globulin concentration among patients with HCC in a recent study [134]. This implies that the much higher prevalence of anti-HCV in HCC cases than in controls may not simply be due to hyperglobulinemia. Non-specific reactivity may also arise from prolonged storage of frozen serum samples [142]. However, this may only bias the relative risk of developing HCC associated with anti-HCV status toward the null if the duration of serum storage prior to testing were the same for cases and controls.

The exact meaning of a positive test for anti-HCV is not completely understood. The prevalence of anti-HCV was extremely high in patients with chronic post-transfusion non-A, non-B hepatitis, but it was much lower in those with acute resolving post-transfusion non-A, non-B hepatitis [10]. In a follow-up study of transfusion recipients affected with acute and chronic non-A, non-B hepatitis, anti-HCV persisted in the circulation of most patients affected with chronic hepatitis. But the patients affected with acute resolving hepatitis did not proceed to anti-HCV positivity after a certain period of follow-up [143]. Blood donors who were positive for anti-HCV usually had no symptoms or signs of liver disease. These persons may also have persistent HCV infection in analogy to the asymptomatic carriers of HBV. It would be of interest to assess the subsequent clinical course of these symptom-free carriers of HCV. Alberti et al. studied liver biopsy samples from individuals who had been anti-HCV positive for more than 6 months but had no symptoms or signs of liver disease [144]. Out of 23 patients, 16 (70%) were found to have histological evidence of chronic hepatitis which could not be predicted by serum ALT, but was well-correlated with HCV-RNA in serum. These findings have challenged the hypothesis regarding the existence of 'true' healthy carriers of HCV.

HCV is a virus containing a positive-stranded RNA genome [145]. The oncogenic potential of HCV is unclear so far. According to currently available data, HCV does not integrate into the host genome like HBV [145]. Insertional mutation is therefore unlikely to be a mechanism responsible for HCV-related hepatocarcinogenesis. HCV infection has been shown to be strongly associated with chronic liver disease and cirrhosis. This suggests that the hepatocarcinogenesis of HCV may be a process from unresolved viral hepatitis, chronic hepatitis, liver cirrhosis to HCC. However, HCV infection may also be associated with HCCs without cirrhosis [123]. The mechanism through which HCV may directly contribute to the development of HCC requires further investigation. The heterogeneity of HCV genome has been demonstrated [145]. Although different HCV strains have been found in Japan and the United States,

whether the different viral strains may play a different role in the pathogenesis of HCC remains unclear.

5. Interactions of HBV or HCV with other risk factors in the etiology of HCC

5.1. Joint effect of HBV and HCV

Interactive effects between HBV and HCV in the development of HCC were recently postulated by several authors on the basis of case-series and case-control studies. Colombo et al. investigated the prevalence of anti-HCV in Italian patients with HCC and chronic liver diseases [122]. The co-occurrence of anti-HCV and anti-HBc was observed in 49 of 91 (54%) HBsAg-negative HCC patients, but only in 26 of 139 (19%) patients with non-A, non-B chronic liver disease. This result implies that infection with both HBV and HCV may cause a more serious liver disease than infection with only one single agent. In a case-control study on the possible association between HCV infection and HCC in the United States including 51 HCC patients (12 black and 39 white) and 128 controls (1 black and 127 white), a synergistic effect of HBV markers and anti-HCV on the HCC risk was observed [137]. In a case-control study involving 185 HCC cases and 432 hospital controls in Greece, the relative HCC risk associated with anti-HCV positivity was substantially higher among HBsAg carriers than among non-carriers (relative risk = 20.0 vs. 4.8) [135]. Two studies conducted in Taiwan also reported a significant interaction between anti-HCV and HBsAg [32,133]. In our previous case-control study, 9 HCC cases out of 127 were positive for anti-HCV and HBsAg but none of the 127 matched-controls were observed to be positive for both viral markers [32]. The other case-control study in Taiwan found that the relative risk of developing HCC was 14.0 for carriers of HBsAg alone, 27.1 for individuals with anti-HCV positivity alone, and 40.1 for those who were positive for both HBsAg and anti-HCV [133]. The prevalence of anti-HCV was about 1% in the general population, 3.5% in prostitutes and more than 80% in hemophiliacs and parenteral drug abusers in Taiwan [111]. As most HBsAg carriers are infected in early childhood in Taiwan [3], it seems unlikely that the co-occurrence of HBsAg and anti-HCV among HCC patients is due to common transmission routes. It is therefore reasonable to speculate that HCV superinfection in HBsAg carriers may synergistically increase the risk of developing HCC.

It has been shown that HCV superinfection suppresses HBV replication in patients with chronic hepatitis B. However, chronic hepatitis B patients with anti-HCV were more likely to have severe liver disease than those without anti-HCV [146]. The mechanism of the interaction between HCV and HBV in the pathogenesis of HCC merits further scrutiny.

5.2. Joint effect of HBV and aflatoxins

Aflatoxins, especially aflatoxin B₁ (AFB₁) which is the most abundant and potent naturally occurring form, are well-established hepatic carcinogens in many animal species [147,148]. Heavy aflatoxin contaminations of food have been reported in tropical Africa and southeastern Asia where carrier rate of HBV is also high. The interaction between aflatoxin exposure and chronic HBV infection has long been suspected to play an important role in the induction of HCC in these areas.

Four ecological correlation studies have assessed the relationship among aflatoxin exposure, chronic HBV infection, and the incidence of HCC [149-152]. In a study carried out in Swaziland, the level of aflatoxin intake was measured in dietary samples from representative households across four broad geographic regions. Aflatoxin exposure was found to be a more important determinant of HCC than HBsAg carrier status [149]. In a study including eight districts of different HCC incidence and ethnic groups in Kenya [150], the aflatoxin exposure measured by urinary excretion of AFB₁-guanine adducts was found to be moderately correlated with HCC incidence in an ethnic group. There was no synergistic effect of chronic HBV infection and aflatoxin exposure on the risk of HCC. In southern Guangxi of China, where the annual mortality rate of liver cancer was as high as 120 per 100 000 population, it was observed that the variation in HCC mortality rate across communities was strongly correlated with the local level of aflatoxin exposure measured by the regular collection and testing of staple foods [151]. However, there was little association between prevalence of HBsAg positivity and liver cancer mortality in these communities. In contrast, no association between HCC mortality and aflatoxin exposure was observed in a study in 48 counties of China. There was a wide range of aflatoxin exposure, HBsAg carrier rate, and HCC mortality rates in these study areas. The HCC mortality was not associated with the aflatoxin exposure indexed by aflatoxin metabolites in pooled urine samples, but it was positively correlated with the HBsAg carrier rate [152]. However, no details on the time of urine collection were stated.

Ecological correlation study evaluates the association between exposure and disease based on data collected at the population level. As the contamination level of aflatoxin in foodstuffs of a given population does not necessarily reflect the exposure to aflatoxin at the individual level, several methods for measuring urinary aflatoxin metabolites and AFB₁-albumin adducts have been applied to quantitate individual AFB₁ exposure [153,154]. In a recent nested case-control study using urinary concentration of AFB₁ metabolites as a biomarker of aflatoxin exposure in Shanghai in China, a strong interaction between chronic HBV infection and aflatoxin exposure was observed [16]. For individuals

who were chronic HBV carriers and had a detectable level of urinary aflatoxin metabolites, the relative risk of developing HCC was 12.5-times higher than those who had chronic HBV infection alone.

The synergistic interaction between HBV and aflatoxin in hepatocarcinogenesis has also been studied in duck and transgenic mouse models. In a Pekin duck model, the incidence of liver tumors (including adenoma and HCC) was higher in ducks infected at birth with DHBV and treated with a dose of 0.08 mg/kg body weight of AFB₁ (3/6) than in the DHBV-uninfected ducks treated with the same dose of AFB₁ (3/10). However, no liver tumors were observed in 13 DHBV-infected ducks treated with a lower dose (0.02 mg/kg body weight) of AFB₁, while 2 of 10 DHBV-uninfected ducks treated with the lower dose of AFB₁ had developed liver tumors [49]. The other Pekin duck model conducted by Cullen et al. showed that the daily administration of aflatoxin for 60 days induced HCCs in 4 of 8 newly hatched Pekin ducks congenitally infected with HBV. HCCs were also observed in 3 of 4 DHBV-uninfected ducks neonatally exposed to aflatoxin [50]. Thus the findings in these duck models did not support an interaction between DHBV and aflatoxin exposure.

In a transgenic mouse model, exposure of female HBsAg transgenic mice to aflatoxin caused more rapid development of premalignant changes and more adenomas and HCCs than in the transgenic mice not exposed to the carcinogen [155].

AFB₁ can bind covalently with cellular DNA [156,157]. It may act as an initiator or a promoter through causing mutations of proto-oncogenes [158,159] or tumor suppressor genes [79,160] which play crucial roles in the pathogenesis of HCC. However, numerous findings from in vitro and in vivo studies suggested that the mammalian liver may be relatively resistant to chemical carcinogenesis in the mitotically inactive situation [161]. A majority of HCCs were also observed to occur in cirrhotic livers [111]. It is therefore reasonable to speculate that regenerative hyperplasia stimulated by chronic HBV infection may have an important role in the aflatoxin-induced hepatocarcinogenesis.

Enhancement of metabolic activation of AFB₁ by HBV infection may be an alternative mechanism for the interaction of AFB₁ with HBV. AFB₁ was activated much more efficiently by liver preparations from WHV-carriers than by those from uninfected animals [162]. A significant enhancement of the AFB₁ activation was also observed in human biopsies from HBsAg carriers with mild chronic active hepatitis [163]. However, this finding was observed on a limited number of human liver tissues. No association of the presence of AFB₁-DNA adducts with either the integration of HBV DNA [164] or the expression of HBV antigens [165] was observed in liver tissues of HCC cases.

5.3. Interaction of HBV with alcohol drinking or cigarette smoking

There was some evidence for interaction of chronic HBV infection with alcohol drinking or cigarette smoking in the development of HCC. In a nested case-control study involving 20 HCC cases identified from a cohort study of 8646 male voluntary blood donors who were found to be HBsAg-positive and 40 age-sex-matched controls selected from the cohort in Japan, a strong dose-response relationship between alcohol drinking and HCC was observed after adjusting for the habit of cigarette smoking. A much higher risk of HCC among heavy smokers was also observed when compared with carriers of HBsAg without a habit of cigarette smoking after adjusting for alcohol drinking [42].

In our previous case-control study of 200 male HCC cases and 200 age-sex-ethnic group-residence-matched community controls in Taiwan, an additive synergism of HBV carrier status with alcohol drinking and cigarette smoking was observed [11]. Habitual alcohol drinking (defined as drinking alcohol more than 3 days per week for more than 15 years) and cigarette smoking were associated with a three- to five-times increase in risk of HCC among chronic HBV carriers. However, no adjustment was made for the effect of one risk factor when the relative risk for the interaction between the other risk factor and HBV carrier status was estimated.

5.4. Interaction of HCV with alcohol drinking, cigarette smoking and hepatitis D virus

There have been relatively few investigations on the interactive effects between HCV infection and other risk factors in the etiology of HCC. A high frequency of anti-HCV has been reported among patients with alcoholic liver cirrhosis and patients with alcoholic cirrhosis com-

bined with HCC [120,121,127]. This suggests that HCV infection may be involved in the development of liver cirrhosis and HCC in some patients with alcohol-induced liver disease. A synergistic effect on HCC development between anti-HCV positivity and habitual alcohol drinking was also observed in our previous case-control study in Taiwan [32]. However, no adjustment was made for cigarette smoking and HBsAg status.

The evidence for the interaction between HCV infection and cigarette smoking in the etiology of HCC was from two case-control studies [32,166]. A case-control study conducted in Greece reported the relative risk for developing HCC in relation to anti-HCV positivity after controlling for age, sex, and HBsAg status was 6.8 (95% CI = 3.6–13.0) among smokers but only 3.2 (95% CI = 1.0–10.2) among non-smokers [166]. In our previous case-control study, the relative risk of HCC was 1.5 (95% CI = 0.8–2.6) among cigarette smokers who were negative for anti-HCV, 5.6 (95% CI = 0.5–286.4) for non-smokers who were positive for anti-HCV, and as high as 13.9 (95% CI = 1.8–630.9) for cigarette smokers who were anti-HCV positive, as compare with non-smokers who were anti-HCV negative [32]. However, alcohol drinking and HBsAg status were not taken into account when these relative risks were calculated.

Cigarette smoke contains many chemical carcinogens associated with tumor initiation and promotion. As liver is the major site for the metabolism of external chemicals, it may also be a target for the action of these carcinogens. A moderate excess risk of HCC among cigarette smokers has been demonstrated in several previous studies [11,13–15]. The mechanism underlying the synergistic effect between anti-HCV positivity and cigarette smoking remains to be studied.

Possible interactive effect between HCV and hepatitis

Table 6

Summary of associations of HCC risk with HBV or HCV/non-A, non-B post-transfusion hepatitis by different study designs

Study design	HBV ^a	HCV/non-A, non-B hepatitis
Ecological	Positive association internationally [21] and intranationally [3,22].	
Migrant	Chinese migrating to Singapore and the United States retain a high risk of developing HCC [20].	
Case-control	Strong positive association; relative risk associated with HBsAg status ranging 5–20 (11,27–38).	Most studies suggested strong positive association with a relative risk of anti-HCV positivity ranging 6–62 [32, 132–138]. One study in Thailand reported no association [33].
Cohort	Strong positive association; the relative risk associated with HBsAg status ranged 6–100 [15,39–43].	Only one nested case-control study has shown positive association [17].
Clinical/experimental	Hepadna viruses share certain important features with retroviruses in molecular structure and replication mechanism [167]. HBV DNA integrates into host genome [56–68]. HBV X and pre S2/S gene products have a transactivation function [89–98,104,105]. Hepadna viruses can induce HCC in animal models [46,47]. HBV transgenic mice have a much higher risk of HCC than non-transgenic mice (53,54).	Several cases develop HCC after non-A, non-B post-transfusion hepatitis [5–8]. Liver cell dysplasia is associated with non-A, non-B hepatitis [116]. A high frequency of antibody to HCV was detected in patients with various chronic liver diseases [111, 118–128]. A case of HCC was reported in a chimpanzee 7 years after inoculation with human plasma from a patient with non-A, non-B hepatitis [168].

^aReference numbers shown in square brackets.

D virus (HDV) in the etiology of HCC has been investigated in only one case-control study [166]. No evidence of synergistic effect between anti-HCV and anti-HDV was found in this study. However, there were only 9 HCC cases positive for anti-HDV.

6. Conclusions

The associations of HCC with HBV and HCV/non-A, non-B hepatitis in different study designs are summarized in Table 6. As can be seen, the association between HBV and HCC is strong and consistent, and has been demonstrated using various study designs. However, the evidence for the association between HCV infection and HCC is limited to the findings from case-series and case-control studies. Only one nested case-control study conducted in a limited area and one animal study based on a follow-up of a limited number of chimpanzees have investigated the role of HCV in the causation of HCC. Therefore, efforts should be made to carry out further prospective studies on various populations and develop additional animal models for the study of HCV-related hepatocarcinogenesis. On the other hand, there has been considerable evidence suggesting that HCC is multifactorial in origin. Either HBV or HCV may not be a sufficient cause for the development of HCC. However, only very few studies have been conducted to assess the HCC risk associated with joint exposures to HBV or HCV and to other major risk factors of HCC, especially aflatoxins. The interactive effects between these viruses and other risk factors in most previous studies were not adequately explored either. Further studies aimed at the investigation of these interactive effects may improve our understanding of the multifactorial etiology of HCC.

Reviewer

This paper was reviewed by David Milich, Ph.D., Department of Molecular Biology, Scripps Research Institute, La Jolla, CA 92037 USA.

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Biographies

Ming-Whei Yu received a Ph.D. degree from the College of Medicine, National Taiwan University. She completed her post-doctoral training in the Division of Environmental Sciences, School of Public Health, Columbia University. She is currently the Associate Professor in the Institute of Epidemiology College of Public Health, National Taiwan University. *Chien-Jen Chen* received a Sc.D. degree from the School of Hygiene and Public Health, Johns Hopkins University. He was an International Fogarty Research Fellow in the Division of Environmental Sciences, School of Public Health, Columbia University. He is currently the Professor and Director Institute of Epidemiology in the College of Public Health, National Taiwan University and a Fellow of the American College of Epidemiology.