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An update on hepatocarcinogenesis

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Hepatocellular carcinoma is the most common cancer occurring in males in the world and has an annual incidence worldwide of 1 million. There are variations in its geographical distribution. It is the second most common cancer in Southeast Asia, including Hong Kong and ranks third among males in China.

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Introduction

Epidemiological and laboratory investigations have established a strong and specific association between chronic hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC).¹ Cirrhosis is another major aetiological factor that has been incriminated. Chronic hepatitis C virus (HCV) infection is also thought to be a cause of HCC, especially in Japan.² Environmental factors, particularly chemical carcinogens such as aflatoxin, smoking, genetic predisposition, and sex hormones may act to promote hepatocarcinogenesis. The exact mechanisms of neoplastic transformation are not understood, but alterations in the p53 gene and other genes are now believed to be essential steps.^{3,4} These various factors are discussed in detail (Table 1).

Hepatitis B virus infection

The five criteria for establishing the oncogenic role of any virus, according to Zuckerman,⁵ are: (i) the infection must precede oncogenesis; (ii) the tumour cells must contain antigens specific for the virus; (iii) the tumour cells can produce the viral agent; (iv) the virus

can cause malignant transformation both in vitro (in cell cultures) and in vivo (in animal models); and (v) active immunisation against the virus will reduce the incidence of the tumour.

Evidence for criteria (i) to (iv) have been accumulating through increased knowledge of the epidemiology, histology, molecular virology, and from animal models in relation to HBV infection. A current controlled study in Gambia, where half of the population is being vaccinated with hepatitis B vaccine,⁷ may supply the definitive support for criterion (v).

Epidemiology

Geographic parallelism

There is a strong positive correlation between the incidence of HCC and the prevalence of HBsAg carriers.^{1-3,6} Southeast Asia and sub-Saharan Africa have very high HBsAg prevalence rates (more than 10%) and have the highest incidence of HCC while the low incidence of HCC in Northern Europe, North America, and Australia, have correspondingly low HBsAg carrier rates (Table 2).

The close association between HCC and HBsAg carrier rate also applies to areas within a country, e.g. in China, provinces south of the Yangtze river have a higher incidence of HBsAg carriers and HCC than do provinces north of the river.

Hepatitis B virus markers in hepatocellular carcinoma

The incidence of HBV markers, especially HBsAg, is consistently higher in HCC patients than it is in con-

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trol populations from the same area (Table 2). The relative risks and attributable risks estimated from some of the published data are 11 to 12 times and 42% to 49%, respectively (Table 3).⁸⁻¹⁴

Maternal frequency of HBsAg positivity in hepatocellular carcinoma patients

Larouze et al first reported a higher incidence of HBsAg in mothers of HCC subjects when compared with the fathers.¹⁵ This was subsequently confirmed by other studies, including those from Taiwan and Japan. Prospective studies done in Taiwan and Hong Kong show a higher incidence of HBV transmission to infants during the perinatal period from HBsAg-positive mothers.^{16,17} These studies show a higher risk of HCC in subjects who acquire the infection early in life.

Prospective study of risk of hepatocellular carcinoma in HBsAg carriers

The results of five cohort studies that compared the occurrence of HCC in HBsAg carriers with that of a control population are summarised in Table 4.^{12,18-21}

The relative risk of HCC calculated from cohort studies and case control studies are similar with the exception of the study from Taiwan (Tables 3 and 4). From the Taiwan study, it is estimated that the proportion of HBsAg carriers dying from HCC and/or cirrhosis is approximately 25% (50% for male carriers and 14% for female carriers).¹⁸

The very high relative risks of HCC reported, especially for Taiwanese, indicate a strong and consistent association between HBV and HCC. The lack of association of HBV with other cancers and metastatic liver cancers suggests that this association is also specific.¹²

Histology

In patients with HCC, HBsAg can commonly be demonstrated by histochemical stains or immunological techniques in the cytoplasm of non-malignant hepatocytes.^{22,23} Less frequently, the core antigen (HBcAg) is identified in the nuclei.²⁴ In a survey of 566 necropsy cases of cirrhosis and HCC in Hong Kong Chinese, HBsAg was identified in the liver in 50% of cases of cirrhosis alone, 39% of HCC alone, 83% of HCC with cirrhosis, but only 5% in a controlled series.²⁵ The significantly higher prevalence of tissue HBsAg in cases of HCC with or without cirrhosis supports the hypothesis that HBV is oncogenic.

Even in low incidence areas for HCC, HBsAg can be found in the livers of many patients with this tu-

mour. Nayak et al show that in India, a low incidence area for HCC and HBV infection, HBsAg is demonstrable in more than 90% of cases of HCC.²⁶ The HBV antigens can also be identified in carcinoma cells.^{27,28} This is a comparatively rare finding and suggests that HBV replication in tumour cells may be defective. Those suffering from HCC are in the integrated HBV phase.

Further histological evidence for the role of HBV in hepatocarcinogenesis is provided by the presence of a significant correlation between tissue HBsAg and liver cell dysplasia, which is generally considered a pre-malignant lesion.²⁹

Molecular virology **Hepatoma cell lines**

A number of hepatoma cell lines can successfully produce HBsAg³⁰ and all HBsAg-producing cell lines have been shown to have integrated HBV DNA. Some cell lines not synthesising HBsAg also show HBV DNA integration. The PLC/PRF/5 cell line (Alexander cell line), which has been most thoroughly studied, contains integrated HBV DNA with at least six different sites of integration.³¹ These data indicate that there is an association between HBV and the hepatoma cell lines.

Hepatitis B virus DNA integration into human hepatocellular carcinoma

There is an 11 base pair nucleotide sequence (5'TTCACCTCTGC) that is a direct repeat of each other (DRI and DR2) at both sides of the cohesive ends of the HBV genome. These DRI and DR2 sequences are preferential sites for viral integration into the host genome. Studies show that HBV DNA integration is almost invariably present in HBsAg-positive HCC patients. The integration of HBV DNA in HBsAg-negative patients ranges from 0% to 100%.^{32,33}

The cloning and sequencing of the integrated HBV DNA found in HBsAg-negative HCCs may help explain the discrepancies. Yaginuma et al analysed the integrated HBV DNA in one HBsAg-negative cell line and found a single integrated copy of a subgenomic region of HBV DNA that spanned the middle of the pre-S gene to the end of the X gene.³⁰

The presence of integrated HBV DNA in the non-tumourous parts of HCC-bearing livers further suggests that integration precedes oncogenesis. The use of reverse transcriptase in the replication of HBV raises the issue of oncogenes in the causation of HCC. Retroviruses are highly oncogenic through integrations

Table 1. Diseases and conditions associated with hepatocellular carcinoma

<p>Chronic hepatitis</p> <ul style="list-style-type: none"> • Hepatitis B virus • Hepatitis C virus 	<p>Sex steroids</p> <ul style="list-style-type: none"> • Contraceptive steroids • C₁₇ alkyl androgens
<p>Cirrhosis</p> <ul style="list-style-type: none"> • Alcoholic cirrhosis • Autoimmune chronic active hepatitis • Primary biliary cirrhosis • Cryptogenic cirrhosis 	<p>Carcinogens</p> <ul style="list-style-type: none"> • Aflatoxin B₁ • Nitrosamines • Cigarette smoking • Ditch water
<p>Metabolic diseases</p> <ul style="list-style-type: none"> • Haemochromatosis • Alpha-1-antitrypsin deficiency • Wilson's disease • Tyrosinosis • Galactosaemia • Type I glycogen storage disease • Ataxia telangiectasia • Hereditary haemorrhagic telangiectasia • Porphyria cutanea tarda 	<p>Irradiation</p> <ul style="list-style-type: none"> • Thorotrast exposure • Radiation
	<p>Miscellaneous</p> <ul style="list-style-type: none"> • Obstruction of inferior vena cava • Immunosuppression after renal transplant

to specific oncogenes. In contrast to retroviruses, HBV shows a lower affinity for integration and hence a longer infection time is usually required for integration to occur. Furthermore, different integration sites have been identified in different HCCs.

The role of the X gene

The X protein, the product of the X gene, is expressed in the liver of HBV carriers and its antibody is present in the serum of some HBV-infected individuals, especially those with HCC. Moriarty and co-workers evaluated 254 serum samples and found antibody to X protein in: 5.8% of asymptomatic HBV carriers; 13.4% of those with HBV-related chronic hepatitis; 15.7% of individuals with HBV-related cirrhosis; 72.7% of those with HBV-related cirrhosis and HCC; and in none of the healthy controls.³⁴

The relationship between the antibody to the X protein and the role of the X protein in hepatocarcinogenesis requires further elucidation. In tissue cultures, X protein acts as a transcriptional transactivator of viral genes. It is possible that this protein may alter host gene expression in such a way as to lead to malignant transformation of hepatocytes. Transgenic mice harbouring the X gene develop multifocal areas of altered hepatocytes, benign adenomas, and finally, HCC, akin to the sequence of events

seen in chemical carcinogenesis. The X protein is expressed in a particular subset of hepatocytes and results in focal rather than generalised liver pathology.³⁵ These findings support the view that HBV is directly carcinogenic, and that chronic necroinflammatory liver disease is not an absolute prerequisite for hepatocarcinogenesis.

Summary

The World Health Organization (WHO) has reported on the significance of HBV DNA integration and its relation to the development of HCC.¹ The following quotation from the WHO Technical Report Series concludes: "All the published evidence is consistent with the interpretation that integration of HBV DNA into the genome of the hepatocyte precedes the development of hepatocellular carcinoma by months or years ... Although these studies do not prove that hepatitis B virus is oncogenic, the finding of HBV DNA in many patients with hepatocellular carcinoma and in all patients with hepatocellular carcinoma with markers of the virus is highly suggestive."

Animal models

Three animal viruses are phylogenetically related to the human HBV, all being members of the genus hepadnavirus (Table 5). These are found in: (i) the eastern woodchuck (*Marmota manax*) [WHV], (ii) Beechey

ground squirrel (*Spermophilus beecheyi*) [GSHV], and (iii) Pekin duck (*Anas domestica*) [DHBV].

Persistent antigenaemia of all three viruses are associated with the development of HCC in their respective hosts (Table 5). Furthermore, it was found that woodchucks, raised in the absence of external co-carcinogens, all developed HCC. This means that at least one of these hepadnaviruses is capable of inducing hepatocarcinogenesis in woodchucks without any co-carcinogens. It has been suggested that the development of HCC in these woodchucks may be related to the necroinflammation caused by WHV.

A more recent study³⁶ shows that even in woodchucks with antibodies against WHV (considered to have recovered from acute HBV infection), HCC can develop 20 to 30 months afterwards. The majority of these woodchucks have low levels of integrated WHV DNA in the tumour and in non-tumourous tissues. By

analogy, this may account for the development of HCC in human subjects who have "recovered" from acute HBV infection with the development of anti-HBs and/or anti-HBc.

Evidence that factors other than hepatitis B virus are involved

While the evidences presented above is supportive of the causal role of HBV in HCC, there are several loop-holes that suggest that HBV is not the sole (and in some areas like Japan, not even the main) causative agent for HCC.

Firstly, a proportion of HCC patients are negative for HBV serological markers; molecular virological studies show no HBV DNA integration in the genome of their HCCs. Secondly, the incidence of HCC is low in Greenland Eskimos, despite a very high HBsAg carrier rate, so chronic HBsAg antigenaemia alone does not lead to HCC. Thirdly, the frequency of HCC has

Table 2. Death rates from hepatocellular carcinoma, and incidence of HBsAg positivity in the general population and in hepatocellular carcinoma cases by country/place

Country/Place	Standardised death rate (HCC) per 100 000 in males	HBsAg carriers in general population (%)	HBsAg positivity in HCC (%)
Hong Kong	38.9	10.0	82.0
Singapore (Chinese)	31.0	6.6	35.3
China	19.6	14.5	86.0
South Africa (Bantu)	19.2	9.0	61.6
Greece	16.8	4.7	63.1
Japan	12.5	2.6	40.3
Italy	10.7	3.0	66.0
Spain	10.4	0.3	35.0
Uganda	6.5	6.5	55.2
Thailand	4.1	9.8	71.4
India	3.1	3.2	41.2
Greenland		2.4*	10.0 [†]
Britain	2.0	1.4	47.4
Australia	1.4	0.2	40.0
United States.	1.4	0.3	17.0
Philippines	-	15.5	70.0
Senegal	-	11.7	61.2
Taiwan	-	12.5	70.3

* standardized death rate for primary liver cancer, which included hepatocellular carcinomas, cholangiocarcinoma, and hepatoblastomas;

[†] between 10-20 years old

Table 3. Case-control studies on HBsAg and hepatocellular carcinoma

Study population	No. of subjects		HBsAg+ (%)		Relative risk	Attributable risk (%)
	HCC	Control	HCC	Control		
Hong Kong	107	107	82.0	18.0	21.3	78.5
China	50	50	86.0	22.0	17.0	77.9
United States	86	161	17.9	0.0	17.0	-
South Africa	289	213	61.6	11.3	12.6	56.7
Senegal	165	328	61.2	11.3	12.4	56.3
Philippines	104	84	70.0	18.0	10.83	63.9
Greece	184	451	45.9	7.3	10.7	41.6

tripled during the past 15 years in Japan, even though the prevalence of HBsAg antigenemia there is declining; HCV is the agent incriminated. Fourthly, the comparative young age of HCC in Africans compared with Asians, raises the question of environmental factors such as a co-factor/co-carcinogen being present in rural areas. Fifthly, in a prospective study of 613 HCC patients in the United Kingdom, cirrhosis, but not HBsAg positivity, was the major risk factor for the development of HCC.³⁷

Cirrhosis

Cirrhosis of different aetiology can be complicated by HCC, and 60% to 90% of HCC occurs in cirrhotic livers.^{25,38} Macronodular postnecrotic cirrhosis carries the greatest risk of HCC. It has been shown that South Africans with macronodular cirrhosis are at a higher risk for developing of HCC than are Caucasians with micronodular cirrhosis.

It is thought that HCC development following cirrhosis is the result of regenerative and proliferative processes that accompany chronic necroinflammatory changes. This is supported by the finding that necroinflammation in WHV-infected woodchucks precedes the development of HCC.³⁶ Alternatively, cirrhosis of the liver may alter the metabolism of environmental carcinogens and thus enhance HCC development.

Most HCC in Caucasians is associated with alcoholic cirrhosis. Zaman et al³⁷ conclude that cirrhosis per se is the main risk factor in the development of HCC and that HBsAg carriage plays a role only by virtue of its cirrhogenic effect. For the 21% HBsAg-positive cases, chronic HBV infection was less important than alcohol as the cause of cirrhosis. This study includes follow up of only those patients with known cirrhosis, and patients who presented with HCC pri-

marily, with no preceding symptomatic cirrhosis, were excluded. It is well documented that the majority of HCC is associated with asymptomatic cirrhosis.³⁸ The validity of the conclusion of this study should therefore be taken with reservation.

In patients who are HBsAg-positive, however, there are two pieces of evidence that cirrhosis probably plays a role in the causation of HCC by the mechanisms suggested above. Firstly, in the Taiwan study of Beasley et al¹⁸ dividing the HBsAg carriers into those with and without cirrhosis, the relative risks of developing HCC in the two groups were 960 and 201, respectively. Secondly, in the necropsy study from Hong Kong,²⁵ it was calculated that the risk factors for HBsAg carriers compared with non-carriers for developing HCC alone was 6:1; for cirrhosis alone, it was 16:1; and for HCC combined with cirrhosis, it was 50:1. In both studies, the presence of cirrhosis increased the relative risk of developing HCC in HBsAg-positive individuals.

Further evidence for the importance of cirrhosis in hepatocarcinogenesis is that in haemochromatosis, HCC usually only develops in those subjects who have cirrhosis.

To conclude, alcoholic micronodular cirrhosis and haemochromatosis are the most common conditions associated with HCC in areas where it is uncommon. The majority of HCCs, however, are accompanied by HBV-related and HCV-related cirrhosis, indicating that HBV and HCV, possibly with cirrhosis as an added factor, are the most important aetiological associations worldwide.

Chronic hepatitis C infection

It is well documented that acute post-transfusion non-A non-B hepatitis (NANBH) progresses to chronic

Table 4. Cohort studies on HBsAg carriers and the risk of hepatocellular carcinoma

Study population	Cohort		HCC risk	
	Total	HBsAg+	Relative risk	Attributable risk (%)
Taiwan	22 707	3454	217.0	93.9
England and Wales	-	3934	42.0	-
New York, USA	-	6850	9.7	-
Japan	32 177	496	10.4	12.7
Osaka, Japan	-	8646	6.6	-

hepatitis in up to 70% of subjects. There have been case reports of post-transfusion NANBH progressing to HCC.³⁹ In a recent analysis of HCC in Japan, Okuda and co-workers demonstrated a sharp rise in incidence of HCC from 1968 to 1984; this was associated with a drop in HBsAg positivity in HCC patients from 50% to less than 30% but 40% of those with HCC in Japan have a history of past blood transfusion with an average time lapse of 20 years.⁴⁰

The strongest evidence incriminating HCV comes from Japan where HCV is probably more important than HBV as a cause of HCC. Multivariate analysis from Japan shows that patients with HCV infection have a greatly increased risk of developing HCC. From 70% to 94% of HBsAg-negative cirrhotic patients with HCC are anti-HCV positive.⁴²

Three patterns of association between HBV and HCV with HCC have emerged. In Chinese and black African patients, HBV is the main risk factor, with HCV having a minor role. In Japan, Italy, and Spain, the opposite is true, with HCV being present in more than 50% of HCC patients.^{2,40-42} In countries with low incidences of HCC, it is likely that both HBV and HCV play a minor role.

The hepatocarcinogenic mechanism for HCV is speculative. Being a RNA virus it does not integrate into chromosomal DNA. It is likely that it induces HCC by causing chronic necroinflammatory liver disease. In the majority of reports, all patients with HCV-related HCC have co-existing cirrhosis of the liver.^{41,42}

Aflatoxins

These important mycotoxins are toxic metabolites of the fungus, *Aspergillus flavus*. There are four major mycotoxins: aflatoxins B₁, B₂, G₁, and G₂; aflatoxin B₁ is the most potent carcinogen. They are metabo-

lised in the liver to active compounds that then bind to the DNA molecules. The *ras* oncogene is known to be activated in aflatoxin-induced HCC in the rat.⁴³

The results of correlation studies that look at the dietary intake of aflatoxin B₁ and the incidence of HCC are shown in Table 6.^{37,43-46} All studies show a direct positive correlation between aflatoxin B₁ exposure and the occurrence of HCC, but as the methods used to estimate aflatoxin B₁ exposure vary, comparisons between these studies are difficult. Two case-control studies that have conflicting results have also been reported.^{8,47} It is generally believed, however, that aflatoxin B₁ is a co-factor in hepatocarcinogenesis in certain areas of China and Africa.

Cigarette smoking

Several studies have shown an increased incidence of HCC among smokers but no dose-response relationship has been demonstrated.^{20,48} Two case-control studies, one from Hong Kong and another from Greece, also report an increased risk of HCC among smokers who are seronegative for HBsAg.^{8,48} The relative risk of developing HCC in these studies ranges from 2.4 to 7.3. Other studies, however, show no association between smoking and HCC.⁴⁹ More studies are required to define the role of smoking, if any, in the causation of HCC.

Alcohol

There is no experimental evidence that alcohol is carcinogenic. Some studies, however, indicate that heavy alcohol consumption is associated with HCC with relative risks ranging from 3 to 8.^{20,50} A cohort study from Japan has demonstrated a dose-response relationship.

On the other hand, two case-control studies, one from Greece and the other from Hong Kong,^{8,51} show

that alcohol consumption is not related to HCC. In two recent studies from Japan, the conclusions are again diverse.^{52,53} Tsukuma and co-workers conclude that alcohol might be a liver carcinogen only because it is causally involved in the development of liver cirrhosis, but Ikeda and co-workers found alcohol intake associated with HCC independent of cirrhosis. It is likely that alcohol is related to the development of HCC through hepatic cirrhosis and/or when there is co-existing chronic HBV infection.

Other risk factors

Haemochromatosis

Haemochromatosis may be complicated by HCC in 8% to 18% of cases.⁵⁴ Most occur in those who have already developed cirrhosis. The risk of developing HCC appears to be the same for males with haemochromatosis and cirrhosis as it is for male cirrhotics in general. However, HCC has also been reported in pre-cirrhotic haemochromatosis. There is evidence that free iron localised in cellular DNA may cause mutation by generating free radicals.

Alpha-1-antitrypsin deficiency

Both the phenotypes ZZ and MZ of α -1-antitrypsin deficiency have been reported to be associated with HCC. All these patients, however, had cirrhosis. Thus the causal role of α -1-antitrypsin deficiency in HCC has yet to be determined.

Sex hormones

The increased risk of liver adenoma among women taking oral contraceptive pills is well documented, but there is no conclusive evidence concerning the cause-effect relationship between its use and the development of HCC. Guechet et al showed an increase in oestrogens and a corresponding drop in circulating androgens in men with cirrhosis and HCC.⁵⁵ Whether these are related to the cause of HCC or are merely the effect of the liver disease requires further clarification.

Cases of HCC occurring in patients with aplastic anaemia following androgenic anabolic steroid therapy have been reported. These tumours are less likely to metastasize and some may regress with the discontinuation of steroid therapy.

Thorotrast

A few cases of HCC have been reported following the use of thorotrast. Thorotrast has a biological half-life of 50 years and contains thorium, which emits high energy alpha, beta, and gamma rays. Most of the liver tumours are haemangiosarcomas and cholangiocarcinomas. This substance has now been banned because of its potential danger.

Inferior vena cava obstruction

An association between membranous obstruction of the inferior vena cava and HCC has been reported, at-

Table 5. The genus hepadnavirus

Virus	Host	Distribution (%)	Prevalence	Association with HCC	DNA size (base pair)
HBV*	Man	Worldwide	0.1 - 20	Yes	3150
WHV [†]	Marmota manax	Pennsylvania Maryland	30 - 35	Yes	3200 + + +
GSHV [‡]	Spermophilus beecheyi	California	0 - 50	Yes	3200 + + +
DHBV [§]	Anas domesticus	China	1 - 60	Yes	3021 +

* HBV hepatitis B virus

† WHV woodchuck hepatitis virus

‡ GSHV ground squirrel hepatitis virus

§ DHBV duck hepatitis B virus

|| HCC hepatocellular carcinoma

tributable to the impaired metabolism of carcinogens as a result of hepatic congestion.

Chromosomal abnormalities and hepatocarcinogenesis

The above risk factors are related to the high incidence of HCC in different parts of the world. It is generally believed that these risk factors eventually act on hepatocyte chromosomes, resulting in multiple genetic changes. Studies of human HCC involving restriction-fragment-length polymorphism have shown allele losses from chromosomes 4, 5q, 11p, 13q, 16q, and especially from chromosome 17p.

Table 6. Correlation studies between aflatoxin B₁ intake and the incidence of hepatocellular carcinoma

Country	HCC in males (per 100 000/year)	Aflatoxin B ₁ (ng/BW*/day)
Thailand		
Ratburi	6.0	31.48
Songkhla	2.0	5.6
Mozambique		
Massinga	9.1	38.6
Manhica-Magude	12.1	20.3
Inharrime	17.8	86.9
Inhambane	21.8	77.7
Zavala	28.8	183.7
Morrumbene	29.1	87.7
Homoine-Maxixe	47.9	131.4
Swaziland		
High veld	4.39	14.3
Middle veld	10.62	40.0
Lubombo	11.07	32.9
Low veld	23.02	127.1
Kenya		
High altitude	3.11	4.88
Middle altitude	10.80	7.84
Low altitude	12.92	14.81
Transkei	9.1	16.5
*BW body weight		

Mutations of p53, the recessive oncogene or anti-oncogene in the short arm of chromosome 17, are the most commonly recognised genetic changes in human cancer. Bressac and co-workers detected abnormalities of p53 in six of seven human HCC-derived cell lines.⁵⁶ Mutations of p53 have been documented in up

to 80% of HCCs from China^{4,57} and from southern Africa.⁵⁸ The mutation consists of a transversion of G to T or of G to C at the third base of codon 249 in the majority of the tumour studies. The wild type allele of p53 appears to be lost as a result of deletion. Wild type p53 protein has a half-life of 15 to 20 minutes while the mutant proteins have half-lives of several hours. It has been suggested that loss of normal gene function may be a key step in hepatocarcinogenesis.

Aflatoxin B₁ has been shown to bind particularly to G residues in G-C-rich regions; codon 249 appears to be the preferred target. Aflatoxin B₁ causes transversion of G to T almost exclusively.⁵⁹ Aflatoxin exposure thus may be the key factor in hepatocarcinogenesis in a subset of patients.

More recently, loss of one copy of the E-cadheric cell adhesion gene on chromosome 16q has been detected in 85% of HCC patients from China.³ Reduced expression of E-cadherin is associated with dedifferentiation and invasive growth that characterise human cell lines and primary tumours. More chromosomal deletions of significance probably await discovery. The interactions between risk factors and chromosomal deletions may eventually complete the picture of hepatocarcinogenesis.

Summary

Proposed mechanism of hepatocarcinogenesis

Hepatitis B virus is the most important aetiological factor incriminated in hepatocarcinogenesis in Chinese and southern Africans. Hepatic cirrhosis probably also plays an important role.

The exact mechanism of oncogenesis is not known but the following hypothesis is proposed. There is rapid viral replication in acute HBV infection, but viral integration may occur even at this early stage. Most, if not all, of the infected hepatocytes, however, are eliminated and if the patient recovers, there is little risk of developing HCC. However, HBV DNA is randomly integrated into the host genome during chronic HBV infection. The development of cirrhosis may hasten oncogenesis by causing necroinflammation and more frequent random viral integration during cellular regeneration. Viral integration may cause altered gene expression; or it may prediagnose these hepatocytes to transform when stimulated by carcinogens/cofactors like aflatoxin B₁, smoking, and alcohol. In some cases, the important gene alteration may be the result of carcinogens alone, e.g. the action of aflatoxin B₁ on the p53 gene. These altered hepatocytes may become au-

tonomous, form malignant clones of cells and result in the formation of unicentric or multicentric HCC.

Further advances in molecular virology and in the development of animal models will enable the above hypothesis to be confirmed, refuted, modified, or refined.

Perspectives for prevention

What are the prospects for the primary prevention of HCC? Prevention of HBV infection can now be achieved by the available hepatitis B vaccines. To reduce the chronic HBV carrier state, which much more commonly follows HBV infection in children than in adults, universal vaccination of all newborns should be conducted. Where it is financially feasible, mothers should be checked for HBsAg status and children born of HBsAg-positive mothers should be given one dose of hepatitis B immune globulin to increase the efficacy of the hepatitis B vaccine. These measures would help to eradicate those HCCs that are HBV-related. The eradication of hepatitis C-related HCC will have to await the development of a vaccine against HCV. Measures directed at the prevention/treatment of other causes of cirrhosis should also be instituted.

References

- World Health Organization (WHO). Prevention of liver cancer. In: World Health Organization Technical Report Series. Geneva: World Health Organization, 1983.
- Tanaka K, Hirohata T, Koga S, et al. Hepatitis-C and hepatitis-B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer Res* 1991;51:2842-7.
- Slagle BL, Zhou YZ, Birchmeier W, Scorsone KA. Deletion of the E-cadherin gene in hepatitis B virus-positive Chinese hepatocellular carcinomas. *Hepatology* 1993;18:757-62.
- Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature* 1991;350:427-8.
- Zuckerman AJ. Viral hepatitis, the B antigen and liver cancer. *Cell* 1974;1:65-7.
- Chinese Academy of Medical Sciences. Atlas of cancer mortality in the People's Republic of China. China Map Press, Beijing, 1981.
- Ryder RW, Whittle HC, Ajdukiewics AB, Tulloch S, Yvonnet B, Smith PG. Virus vaccine in a case-control investigation of primary hepatocellular carcinoma in West Africa. In: Lam SK, Lai CL, Yeoh EK, editors: Viral hepatitis B infection in the Western Pacific region: vaccine and control. Singapore: World Scientific, 1984:211-4.
- Lam KC, Yu MC, Leung JW, Henderson BE. Hepatitis B virus and cigarette smoking: risk factors for hepatocellular carcinoma in Hong Kong. *Cancer Res* 1982;42:5246-8.
- Yeh FS, Mo CC, Luo S, Henderson BE, Tong MJ, Yu MC. A serological case-control study of primary hepatocellular carcinoma in Guangxi, China. *Cancer Res* 1985;45:872-3.
- Lingao AL, Domingo EO, Nishioka K. Hepatitis B virus profile of hepatocellular carcinoma in the Philippines. *Cancer* 1981;48:1590-5.
- Trichopoulos D, Day NE, Kaklamani E, et al. Hepatitis B virus, tobacco smoking and ethanol consumption in the etiology of hepatocellular carcinoma. *Int J Cancer* 1987;39(1):45-9.
- Prince AM, Szmunes W, Michon J, et al. A case control study of the association between primary liver cancer and hepatitis B infection in Senegal. *Int J Cancer* 1975;16:376-83.
- Kew MC, Desmyter J, Bradburne AF, Macnab GM. Hepatitis B virus infection in southern African blacks with hepatocellular cancer. *J Natl Cancer Inst* 1979;62:517-20.
- Austin H, Delzell E, Grufferman S, et al. A case control study of hepatocellular carcinoma and the hepatitis B virus, cigarette smoking, and alcohol consumption. *Cancer Res* 1985;46:962-6.
- Larouze B, London WT, Saimot G, et al. Host response to hepatitis B infection in patients with primary hepatic carcinoma and their families: a case-control study in Senegal, West Africa. *Lancet* 1976;ii:534-8.
- Lee AK, Ip HM, Wong VC. Mechanisms of hepatitis B virus. *J Infect Dis* 1978;138:668-71.
- Beasley RP, Hwang LY, Lin CC, Ko YC, Twu SJ. Incidence of hepatitis among students at a university. *Am J Epidemiol* 1983;117:213-22.
- Beasley R, Hwang LY. Epidemiology of hepatocellular carcinoma. In: Vyas GN, Dienstag JL, Hoofnagle JH, editors. Viral hepatitis and liver disease. New York: Grune & Stratton, 1984:209-14.
- Iijima T, Saitoh N, Nobutomo K, Nambu M, Sakuma K. A prospective cohort study of hepatitis B surface antigen carriers in a working population. *Gann Monogr Cancer Res* 1984;75:571-3.
- Oshima A, Tsukuma H, Hiyama T, Fujimoto I, Yamano H, Tanaka M. Follow-up study of HBsAg-positive blood donors with special references to effect of drinking and smoking on development of liver cancer. *Int J Cancer* 1984;34:775-9.
- Hall AJ, Winter PD, Wright R. Mortality of hepatitis B positive blood donors in England and Wales. *Lancet* 1985;i:91-3.
- Wu PC. Detection of hepatitis B surface antigen in liver biopsies from 655 Chinese patients in Hong Kong. *Asian J Infect Dis* 1978;2:223-9.
- Ho JC, Wu PC, Gibson JB. Hepatitis B surface antigen in hepatocytes at necropsy: comparison with serologic results obtained postmortem or antemortem. *Arch Pathol Lab Med* 1980;104:255-7.
- Wu PC, Lau JY, Lau TK, Lau SK, Lai CL. Relationship between intrahepatic expression of hepatitis B viral antigens and histology in Chinese patients with chronic hepatitis B virus infection. *Am J Clin Pathol* 1993;100:648-53.
- Gibson JB, Wu PC, Ho JC, Lauder IJ. Hepatitis B surface antigen, hepatocellular carcinoma and cirrhosis in Hong Kong: a necropsy study: 1963-1976. *Br J Cancer* 1980;42:370-7.
- Nayak NC, Dhar A, Sachdeva R, et al. Association of human hepatocellular carcinoma and cirrhosis with hepatitis B virus surface and core antigens in the liver. *Int J Cancer* 1977;20:643-54.
- Wu PC, Lam KC. Cytoplasmic hepatitis B surface antigen and the ground-glass appearance in hepatocellular carcinoma. *Am J Clin Pathol* 1979;71:229-34.
- Wu PC. Patterns of hepatitis B surface antigen: localization in cells of hepatocellular carcinoma. *Arch Pathol Lab Med* 1980;103:165-8.
- Ho JC, Wu PC, Mak TK. Liver cell dysplasia in association with hepatocellular carcinoma, cirrhosis and hepatitis B surface antigen in Hong Kong. *Int J Cancer* 1981;28:571-4.
- Yaginuma K, Kobayashi M, Yoshida E, Koike K. Hepatitis B

- virus integration in hepatocellular carcinoma DNA: duplication of cellular flanking sequence at the integration site. *Proc Natl Acad Sci USA* 1985;82:4458-62.
31. Edman JC, Gray P, Valenzuela P, Rall LB, Rutter WJ. Integration of hepatitis B virus sequences and their expression in a hepatoma cell. *Nature* 1980;286:535-8.
 32. Shafritz D, Shouval D, Shermann HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into genome of the liver cells in chronic liver disease and hepatocellular carcinoma. *N Engl J Med* 1981;305:1067-73.
 33. Brechot C, Degos F, Lugassy C, et al. Hepatitis B virus DNA in patients with chronic liver disease and negative test for hepatitis B surface antigen. *New Engl J Med* 1985;312:270-6.
 34. Moriarty AM, Alexander H, Lerner RA, Thornton GB. Antibodies to peptides detect new hepatitis B antigen: serological correlation with hepatocellular carcinoma. *Science* 1985;227:429-43.
 35. Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis-B virus induces liver cancer in transgenic mice. *Nature* 1991;351:317-20.
 36. Korba BE, Wells FV, Baldwin B, et al. Hepatocellular carcinoma in woodchuck hepatitis virus-infected woodchucks: presence of viral DNA in tumour tissue from chronic carriers and animals serologically recovered from acute infections. *Hepatology* 1989;9:461-70.
 37. Zaman SN, Melia WM, Johnson RD, Portman BC, Johnson PJ, Williams R. Risk factors in development of hepatocellular carcinoma in cirrhosis: prospective study of 613 patients. *Lancet* 1985;i:1357-9.
 38. Lai CL, Lam KC, Wong KP, Wu PC, Todd D. Clinical features of hepatocellular carcinoma: review of 211 patients in Hong Kong. *Cancer* 1981;47:2746-55.
 39. Resnick RH, Stone K, Antonioli D. Primary hepatocellular carcinoma following non-A, non-B post-transfusion hepatitis. *Dig Dis Sci* 1983;28:908-11.
 40. Okuda H, Obata H, Motoike Y, et al. Clinicopathologic features of hepatocellular carcinoma—comparison of seropositive and seronegative patients. *Hepato-Gastroenterology* 1984;31:64-8.
 41. Bruix J, Barrera JM, Calvet X, et al. Prevalence of antibodies to hepatitis-C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989;334:1004-6.
 42. Columbo M, Kuo G, Choo QL, et al. Prevalence of antibodies to hepatitis-C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989;334:1006-8.
 43. Sinha S, Webber C, Marshall CJ, et al. Activation of *ras* oncogene in aflatoxin-induced rat liver carcinogenesis. *Proc Natl Acad Sci USA* 1988;85:3673-7.
 44. Shank RC, Gordon JE, Wogan GN, Nondasuta A, Subhamani B. Dietary aflatoxins and human liver cancer: III field survey of rural Thai families for ingested aflatoxins. *Food Chem Toxicol* 1972;10:71-84.
 45. Peers FG, Bosch FX, Kaldor JM, Linsell CA, Pluemen M. Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland. *Int J Cancer* 1987;39:545-53.
 46. Peers FG, Linsell CA. Dietary aflatoxins and liver cancer: a population-based study in Kenya. *Br J Cancer* 1973;27:473-84.
 47. Bulatao-Jayme J, Almero EM, Castro CA, Jardeleza TR, Salamat LA. A case-control dietary study of primary liver cancer risk from aflatoxin exposure. *Int J Epidemiol* 1982;11:112-9.
 48. Garfinkel L. Cancer mortality in non-smokers. Prospective study by the American Cancer Society. *J Natl Cancer Inst* 1980;65:1169-73.
 49. Austin H, Delzell E, Grufferman S, et al. A case control study of hepatocellular carcinoma and the hepatitis B virus, cigarette smoking, and alcohol consumption. *Cancer Res* 1986;46:962-6.
 50. Yu MC, Mack T, Hanisch R, Peters RL, Henderson BE, Pike MC. Hepatitis, alcohol consumption, cigarette smoking, and hepatocellular carcinoma in Los Angeles. *Cancer Res* 1983;43:6077-9.
 51. Maynard JE, Kare MA, Alter MJ, Hadler SC. Control of hepatitis B by immunization: global perspective. In: Zuckerman AJ, editor. *Viral hepatitis and liver disease*. New York: Alan R Liss Inc., 1988:967-9.
 52. Tsukuma H, Hiyama T, Tanaka S, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993;328:1797-801.
 53. Ikeda K, Saitoh S, Koida I, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993;18(1):47-53.
 54. Blumberg RS, Chopra S, Ibrahim R, et al. Primary hepatocellular carcinoma in idiopathic haemochromatosis after reversal of cirrhosis. *Gastroenterology* 1988;95:1399-1402.
 55. Guechet J, Peigney N, Ballet F, Vanbourdole M, Gibondeau J. Sex hormone imbalance in male alcoholic cirrhotic patients with and without hepatocellular carcinoma. *Cancer* 1988;62:760-2.
 56. Bressac B, Galvin KM, Liang TJ, Isselbacher KJ, Wands JR, Ozturk M. Abnormal structure and expression of p53 gene in human hepatocellular carcinoma. *Proc Natl Acad Sci USA* 1990;87:1973-7.
 57. Scorsone KA, Zhou YZ, Butel JS, Slagle BL. p53 mutations cluster at codon 249 in hepatitis B virus-positive hepatocellular carcinomas from China. *Cancer Res* 1992;52:1635-8.
 58. Bressac B, Kew MC, Wands JR, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991;350:429-31.
 59. Foster PL, Eisenstadt E, Miller JF. Base substitution mutations induced by metabolically activated aflatoxin B1. *Proc Natl Acad Sci USA* 1983;80:2695-8.