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Hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is increasing in many countries as a result of an increase in hepatitis C virus (HCV) infection since World War II. The epidemiology of HCC varies with the global region. There have been conflicting observations from different parts of the world concerning the frequency of HCC in patients who in the distant past had post-transfusion non-A, non-B hepatitis. The genetic basis of hepatocarcinogenesis is still poorly understood. In hepatitis B virus (HVB) associated HCC, codon 249 mutation in the *p* 53 gene seems more related to exposure to aflatoxin B1 than to hepatocarcinogenesis itself. HCC that occurs in children in high HBV endemic regions could be associated with germ-line mutations, but little information is available; not much is known about chemical hepatocarcinogens in the environment other than aflatoxins. The X gene of HBV seems to play an important role in HBV-associated hepatocarcinogenesis. There are preliminary observations on the molecular mechanism of HCV-associated HCC, such as HCV core protein inducing HCC in transgenic mice and the NS3 genome transforming NIH 3T3 cells. Pathological distinction between preneoplastic and very early transformed lesions still depends on classical morphology, and a more genetically oriented differential diagnosis is required. Clinical diagnosis based on modern imaging has improved greatly, but is still unsatisfactory in the differential diagnosis of preneoplastic and early transformed nodules, because the vasculature changes that occur within the nodule are not accurately discerned with the current imaging. Use of sensitive des-γ-carboxy prothrombin (PIVKA II) assay, and lectin affinity chromatography separating HCC specific subspecies of AFP molecules with a more practical biochemical technique will further improve diagnosis. Early diagnosis and transplantation are the best treatment at the moment, but transplantation is not widely available because of the donor shortage. Despite successful resection, the remnant cirrhotic liver frequently develops new HCC lesions, seriously curtailing long-term survival. All-out efforts should be directed to the prevention of HCC, through prevention of viral hepatitis, prevention of acute hepatitis from becoming chronic, prevention of chronic hepatitis from progressing to cirrhosis, and prevention of the cirrhotic liver from developing HCC (chemoprevention). At the moment, very few such studies exist.

Key words: **Adenomatous hyperplasia; Hepatitis B virus; Hepatitis C virus; Hepatocellular carcinoma.**

PRIMARY liver cancer ranks fifth in frequency among all malignancies in the world with an estimated number of 437 000 new cases in 1990 (1). The vast majority of primary liver cancer is hepatocellular carcinoma. HCC is on the increase in many countries, particularly in areas where hepatitis C virus (HCV) infection is more common than hepatitis B virus (HBV) infection (2–6). In contrast, the prevalence of HBV-associated HCC has been rather stable in most parts of the world (7,8), and the increase of HCC is linked to increased chronic HCV infection. It now seems that HCV is more carcinogenic than HBV, judging from the frequency of HCC development among HBV- and HCV-induced cirrhosis (9,10). The molecular mechanism of hepatocarcinogenesis seems to be more complex than that for polyp-based colon cancer (11), and the genetic changes that would precede hepatocarcinogenesis are still poorly understood despite numerous studies on oncogenes, suppressor oncogenes and their mutations in HCC. Histopathologically, preneoplastic changes occurring in the hepatocytes are still ambiguous, and an exact morphologic definition of preneoplastic lesions and early HCC has not yet been made,

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although morphologic transition from hyperplastic nodules to overt HCC has been delineated. Clinical diagnosis of small or early HCC based on modern imaging has improved to the point where further improvement of detection sensitivity would confuse the diagnosis and treatment decision. Although various nonsurgical treatment modalities have been developed and the surgical techniques so much improved that there is practically no operative death, the majority of patients eventually succumb, with the exceptions of a few fortunate transplanted patients. The overall 5-yr survival rate worldwide is only 2% because of the late diagnosis (12). Transplantation during an early stage of HCC is the best treatment, with a remarkable recent result (13), but the demand far exceeds available donors, and a serious search is currently underway for ways to prevent HCC. There has been a surge in research on gene therapy for HCC in recent years, but development is slow and clinically available techniques far in the future. In this chapter, some of the recent important developments and future perspectives will be discussed.

Epidemiology

HCC is increasing throughout the world (2–6). The increase is mainly associated with HCV infection, particularly in Japan (2) where about 80% of HCC cases are now associated with chronic HCV infection. Fig. 1 shows the changes in the relative frequency of cirrhosis and HCC among autopsies that have occurred in the past 40 yr in Japan. The increase in HCC and the decrease in cirrhosis alone at autopsy is the significant phenomenon that has occurred in association with increasing HCV infection. Internationally, there are some conflicting epidemiologic results. A large prospective multicenter study in the USA headed by Seeff (14,15) has for more than 20 yr followed 568 patients who developed posttransfusion non-A, non-B hepatitis and a similar number of controls. Not a single patient has as yet developed HCC. In a study from California in which patients who had chronic posttransfusion hepatitis C were followed, 14 of 131 patients developed HCC after an average lapse of 28 yr from blood transfusion (16). This figure is very similar to that obtained earlier in Japan by Kiyosawa et al. (17), in which 99 patients with posttransfusion HCV infection that became chronic were followed. HCC started evolving after a lapse of 15 yr or longer with an average interval of 29 yr. These studies are not quite comparable because of the different patient selection, but there is certain difference between the study in Japan and the one led by Seeff in the USA. In view of the very high percentage of chronic hepatitis that occurs following acute hepatitis C (18), a considerable proportion of Seeff's patients should have developed chronic progressive hepatitis C, and possibly some HCC. In Ireland, anti-D immunoglobulin contaminated with HCV was given to a number of women in 1977 and 1978. In 1994, 704 of these women were found to have positive tests for serum HCV RNA, and 374 of them were studied in March 1997, after a lapse of 20 yr from infection. Serum ALT was slightly elevated in 47%, biopsy showed mild inflammation in 98%, but only 7 women (2 alcoholic) had probable or definite cirrhosis (19). Although this study included only women, who are less prone to develop progressive liver disease than men, the difference between the studies in Japan and studies in the USA and Ireland is striking. Sex and genotype differences alone do not explain it. There may exist genetic and environmental differences currently not understood.

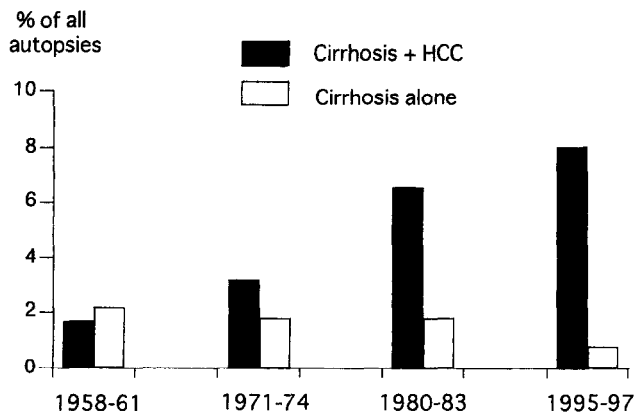

Fig. 1. Temporal changes in the frequency of cirrhosis alone and cirrhosis complicated by HCC at autopsy in Japan (National Autopsy Registry). Before 1960, the relative frequency of cirrhosis alone and cirrhosis complicated by HCC was about the same (1:1). HCC progressively increased, and in 1995–97 the ratio of cirrhosis alone to cirrhosis+HCC became 1:9.

HCV is often transmitted iatrogenically. There are a number of isolated towns in Japan where the anti-HCV positivity among the residents far exceeds that in neighboring towns. This was due either to unsanitary medical practice or to acupuncture carried out by a particular physician or a practitioner (20,21). Among the anti-HCV positive residents of advanced age in such towns, the incidence of HCC is also high. In the Middle East, a similar situation prevails in which HCV is transmitted by the physician. About 24% of the people in Egypt are estimated to carry HCV, and more than 50% of blood donors have anti-HCV in some towns (22,23). These high infection rates in Egypt are linked to the past practice of parenteral antischistoso-

miasis therapy and prevention during 1918–1970 (22). Epidemiologic follow-up of such people will soon provide information and clues to these differences between Japan and USA/Ireland.

Genetic basis of hepatocarcinogenesis

It is known that carcinogenesis is a multistep process (24,25) in which a number of mutational genetic alterations occur, as elegantly demonstrated by Fearon & Vogelstein in the transition of adenomatous polyp to colon cancer: mutation and loss of APC, MCC, altered DNA methylation, *K-ras* mutation, loss of DCC, mutation of *p* 53 and several other alterations (11,26). These involve activation, overexpression or both of oncogene products, and inactivation or loss of tumor suppressor gene products. Compared to colonic mucosa cells, hepatocytes have a great deal more metabolic functions affected by many more expressed genes, and much more complicated genetic alterations are expected to occur in the process of carcinogenesis. There have been many studies in which various oncogenes, antioncogenes, and loss of heterozygosity of chromosomes were found to have a variety of changes (27), but as yet no consistent sequence of genetic changes has emerged. If a uniform sequence of genetic alterations is found, it will open up a new avenue for the identification of the stage of chronic B and C diseases; how close or how far to malignant transformation. It will also greatly assist in the histochemical distinction of cells already transformed and cells that are still able to remodel and differentiate back to the early altered cell state (28).

Earlier, it was found that *p* 53 gene was mutated at codon 249 in HCC from patients in southern Africa (29) and Qidong, China (30), and an association of this particular mutation and aflatoxin B1 exposure was suggested. This mutation is not found in HCC patients in the USA (31), Japan (32), Germany (33), UK (34), Europe outside UK and Germany (35) and Australia (36), and in only a small percentage of patients in Korea (37) and Taiwan (38). In HCC, *p* 53 changes are recognized only in an advanced stage, not in early HCC (39), and clearly it is not a prerequisite for malignant transformation. In an *in vitro* study with a liver cell line, codon 249 mutation of the *p* 53 gene served as a fingerprint for aflatoxin B1-induced hepatocellular carcinoma, but it was not by itself sufficient to immortalize human liver cells (40). Using HBsAg transgenic mice and *p* 53 null mice, Ghebranious & Shell (41) demonstrated that mutation *p* 53ser246, which is equivalent to *p* 53ser249 in man, enhances aflatoxin-induced hepatocarcinogenesis in the absence of HBsAg. However, there is indirect evidence that HBsAg expression and chemical carcinogens exert synergistic effects on hepatocarcinogenesis (42). Thus, it seems that codon 249 mutation is somehow closely associated with exposure to aflatoxin B1, but does not exert a strong transforming impact on the hepatocyte by itself. Aquilar et al. (43) found G-to-T transversion in codon 249 in the nonmalignant liver from patients with HCC from Qidong, the region where HCC incidence is the highest in the world; none of the adjacent nontumorous liver tissues of Korean patients had the same transversion (37). Thus, it is likely that in these patients who are constantly exposed to aflatoxin in Qidong and southern Africa (44), the codon 249 mutation is a germ-line genetic change. Perhaps they need fewer genetic alterations for hepatocarcinogenesis and are closer to carcinogenesis compared with those who do not have this germ-line mutation.

One important aspect that requires more attention is that HCC occurs in young children in areas where HBV infection is endemic such as China and Taiwan, and that childhood HCC is invariably associated with HBV infection (45–47). In Taiwan, for instance, nearly one quarter of males of the third decade are HBsAg carriers (48). Those carriers have acquired the carrier state by vertical transmission from the mother, and the mother-to-child transmission has been going on for generations within the family. It is likely that the germ-line genetic changes induced by HBV infection exist in the parents, and that at birth the child has already inherited these mutations that are required for malignant transformation. Those children are already in a premalignant state, so to speak, which requires only one or few additional genetic changes for hepatocarcinogenesis. In Taiwan, a universal vaccination program was initiated in 1984, and in 10 yr, the carrier rate has dropped from 10% to less than 1% (49). Surprisingly, the incidence of childhood HCC has already declined (50), a remarkable achievement. The premise is that a child born to a carrier mother and immediately vaccinated, and a child similarly born but not vaccinated, will fare differently; the latter will have HBV infection postnatally, which induces genetic changes necessary for hepatocarcinogenesis. Again, HBV infection or the presence of continuous inflammation in the liver favorably acts for carcinogenesis (51,52).

In Switzerland, Chaubert and associates (53) found germ-line mutations of the $p16^{INK4}$ (MYS1) gene, a newly discovered tumor suppressor gene, in adult cases of HCC, suggesting the existence of familial HCC that involves this gene. Four of 26 adult patients with HCC had germ-line point mutations of the $p16^{INK1}$(MTS1) gene, suggesting a familial HCC involving a defect in

this gene. Three of these four did not have cirrhosis. A recent report from Alaska suggested the existence of familial inheritance of HCC in its population (54). Although the concept of familial inheritable HCC is new and requires confirmation, if it does exist, it will provide a very useful tool for the elucidation of genetic changes required in hepatocarcinogenesis. As already noted, there is an epidemiologically interesting area near Shanghai called Qidong. The eastern part of this province is newly reclaimed land from the Yellow Sea where fresh water is difficult to obtain. Among the farmers who have settled there and who drink stagnant ditch or pond water (after boiling), the incidence of HCC is exceedingly high, higher than that in Mozambique. It was thought earlier that carcinogenic pesticides used for farming had contaminated the drinking water, which would account for the high HCC incidence (55). But analysis of organic pesticides contained in fatty tissue of cadavers show no difference between patients who died from HCC and non-HCC patients. There was no indication that aflatoxin exposure was high among ditch water drinkers. It was subsequently found out that a newly identified carcinogen "microcystin", a blue algal hepatotoxin, is the carcinogen that could account for the high HCC incidence among the ditch water drinkers (56). With government subsidies, these farmers have dug deep wells, and the switch to deep well water for drinking has effected a slow but definite decline in HCC incidence (57). These studies have shown that beside aflatoxin B1, there are as yet unidentified chemical carcinogens or mycotoxins in our environment, and exposure to some of them could occur through eating/drinking. Dioxin has been found in maternal milk at a concentration 25 times the permissible level in Japan. Little is known about the hepatocarcinogenic potential of this carcinogen.

Before the discovery of HCV, there was a suggestion that chemical carcinogenesis and virus-induced carcinogenesis are different from the molecular point of view, because integration of HBV DNA into host DNA involves double stranded DNA, whereas chemical carcinogenesis perhaps involves just one strand at a time (58). However, we now know that HCV is even more closely associated with HCC than HBV, and that this virus genome is not integrated into host DNA. One wonders whether there is any basic difference between virus-induced and chemically induced hepatocarcinogenesis. The concept of "initiation" in carcinogenesis was developed from the studies of skin cancer, and was substantiated in animals with chemical carcinogens. It is still unclear whether hepatitis virus infection is the initiator or initiation has already begun when infection occurs with HCV, and whether viral infection or the subsequent inflammation acts as a promoter. The vertically transmitted HBsAg carriers had HBV infection at birth. Then, what is initiation in such individuals? If genetic alterations are the basic carcinogenetic process, chemical carcinogens and viruses may simply act as an inducer of genetic alterations, although the alterations they produce may differ. Another question is how strong the virus-induced inflammation is as a promoting factor in carcinogenesis. These questions will be unraveled in due course.

In the 1980s, many investigators studied the carcinogenetic role of HBV, particularly the integration pattern of viral DNA into hepatocyte DNA, with an assumption that it triggers the oncogenetic process. The woodchuck model (59) demonstrated that integration of viral DNA acts as insertional mutagens (60). Although integration is totally random with respect to the integration site and integrating DNA segments (27,61), it may play a similar role in human hepatocarcinogenesis. More recently, attention has been focused on the X gene of HBV, which is a transactivator upregulating the expression of other viral genes and some cellular promoters such as *c-myc* and *c-jun* (62). The HBV X gene is mutated in some human HCC tissue (63). Kim and his associates (64) succeeded in producing HCC in transgenic mice after placing the entire HBV X gene under its own regulatory elements directly into the germline of mice. Beginning with multifocal areas of altered hepatocytes, followed by the appearance of benign adenomas, the histopathologic changes proceeded to malignant carcinoma. It took more than 8 months for HCC to develop. The interesting question is what happened during the progressive histopathologic changes in terms of genetic alterations, which were perhaps accelerated by the transactivating activities of the X-gene.

The clinical and epidemiologic studies suggest that HCV is perhaps more hepatocarcinogenic than HBV; a much greater proportion of patients with C-cirrhosis develop HCC than do those with B-cirrhosis (7,9). There is a recent report that HCC developed in an allograft within 7 yr from transplantation in a recipient who had had C-cirrhosis (65). HCV is an RNA virus not capable of inducing integration of its genome into the host chromosomal DNA. The HCV genome is translated into a 3000 amino acid polyprotein precursor split into a number of proteins with different functions (18). As yet, none of them has been found to be linked to HCC through a known molecular mechanism. Koike et al. (66) established transgenic mouse lines in which HCV envelope proteins are efficiently expressed in the liver, but no pathologic changes occurred, unlike the surface antigen of HBV expressed in

transgenic mice, which causes liver cell damage (51,52). The titer of the antibody to nonstructural protein NS3 is correlated with the severity of chronic hepatitis C (67). Sakamoto et al. (68) transfected NIH 3T3 cells with an expression vector containing cDNA for the 5′-half or 3′-half sequence of the genome encoding NS3. Only the cells transfected with the 5′-half cDNA rapidly proliferated, lost contact inhibition, grew anchorage independently in soft agar, and formed a tumor in nude mice. Based on these results, they postulate that the 5′ region of the genome segment that encodes NS3 is involved in cell transformation. HCV core protein is an unglycosylated protein and has transcriptional regulatory functions on different cellular genes such as c-myc (69) and the interferon-β gene (70). In this respect, the core protein may be a counterpart of the X gene of HBV. Koike and his group established transgenic mouse lines carrying the HCV core gene and found that the HCV core protein induces hepatic steatosis, as occurs in human liver with chronic hepatitis C (71). When these mice were followed for more than 16 months, the liver first developed adenomas containing fat droplets in the cytoplasm, and then a poorly differentiated HCC evolved within the adenomas (72), presenting the "nodule-in-nodule" feature typically seen in early human HCC in an adenomatous hyperplastic nodule (73). Again, during the precancerous 16 months, a number of genetic alterations must have occurred, and without elucidation of these molecular events, no real information may be gained.

Pathology

With the advent of real-time ultrasonography, small HCCs and nodular lesions in cirrhotic liver came to be readily recognized and resected in increasing numbers in Japan and elsewhere. Arakawa et al. first reported in 1986 (74) that an early HCC evolved in an adenomatous hyperplastic nodule (Fig. 2). Subsequently, a large number of histopathologic studies were conducted with resected livers in this country, and the pathologists are currently in agreement that histologic changes preceding malignant transformation usually occur within dysplastic nodules. These include an increase of cellularity (nuclear crowding), an irregular thin-trabecular pattern with frequent acinus and pseudogland formations, fatty and/or clear cell changes (75–79), and cell invasion within the fibrous stroma and vessel walls (80) in cirrhotic livers. The early HCC is usually well differentiated, and within it evolves less well differentiated HCC. Early lesions of 1–2 cm in size are not encapsulated. It has been shown that many HCCs arise as a tumor of a well differentiated type that contains portal tracts and has indistinct margins (81,82). Rare cases of early HCC not arising within a dysplastic nodule have also been reported with a biopsy specimen (83). These Japanese investigators used the term, "adenomatous hyperplasia" and "atypical adenomatous hyperplasia" to denote preneoplastic nodules without a clear definition. An international committee on the nomenclature of nodular lesions recommended the use of "dysplastic nodule" instead (84,85). Observations similar to those in Japan have since been made in western countries with cirrhotic explants which were used for the study of early HCC and macroregenerative nodules (86–89). Takayama et al. followed 20 patients with dysplastic nodules for up to $4\frac{1}{2}$ yr, and during the follow-up one half of the lesions turned malignant (90). Studies in

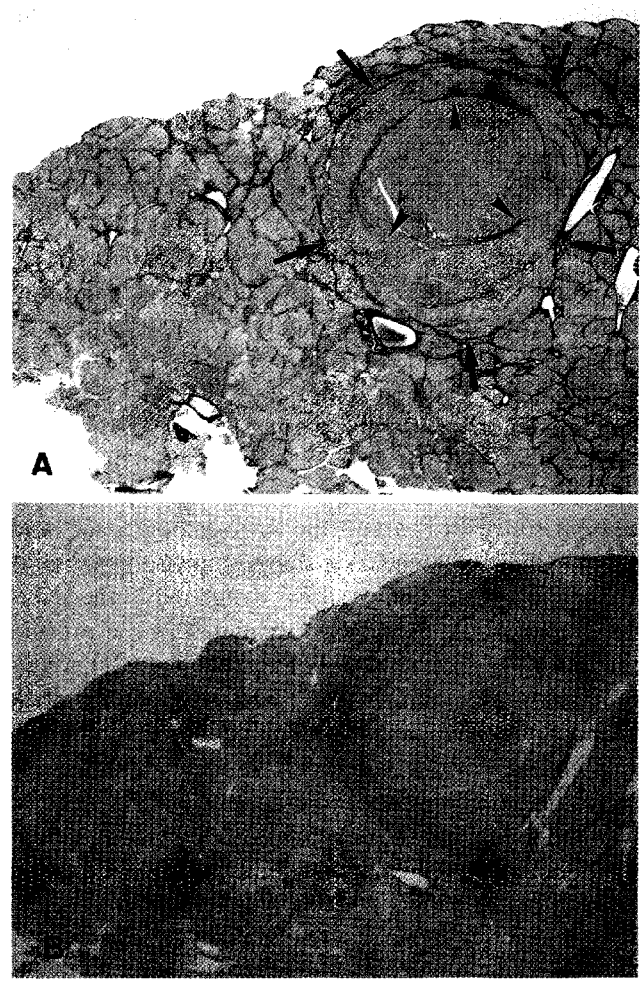

Fig. 2. Early HCC evolving within an adenomatous hyperplastic (dysplastic) nodule, the "nodule-in-nodule" feature. (A) H & E stain, low magnification. Arrows point to the dysplastic nodule and arrowheads, evolving early HCC. (B) Berlin-blue stain. The iron positive dysplastic nodule contains an iron negative early HCC. (Courtesy of Prof. Masamichi Kojiro).

Italy also suggested adenomatous hyperplasia to be nearly an equivalent of early HCC (91,92). Another way of determining the stage in the transition of a preneoplastic lesion to early well-differentiated HCC is to study the temporal vascular changes. It is known that overt HCC is totally dependent on arterial supply. Nakashima et al. (93) showed that as small HCCs increase in size and become increasingly differentiated, the number of portal tracts apparently decreases and intratumoral arterioles develop. These findings may be reflected in the changes of hemodynamics during malignant transformation. Ueda et al. (94) studied 43 ordinary adenomatous hyperplasias, 20 atypical nodules, and 30 early HCCs, and measured cumulative areas of arterial and portovenous lumina in the nodule in comparison with those in the surrounding tissue, and showed that there occurs a sharp stepwise increase of abnormal arterial luminal areas compared to the arteries in the surrounding areas calculated as the ratio in the following order: ordinary hyperplasia (0.21), atypical hyperplastic nodule (0.47) and HCC (0.94). Such changes are also demonstrated by modern imaging with contrast media (95). Based on these and other observations, pathologists and clinicians alike have come to wonder whether the adenomatous hyperplastic nodule itself is already neoplastic, although some express reservations (79,96). Eguchi et al. (97) studied 30 large regenerative nodules, 12 adenomatous hyperplasias, 2 atypical adenomatous hyperplasias and 5 adenomatous hyperplasias containing HCC. They accounted for 43% of the adenomatous hyperplasias found in the vicinity of the 16 resected HCCs. Atypical adenomatous hyperplasia measured 15.8 mm on average, significantly larger than 10.1 mm of adenomatous hyperplasia, and all adenomatous hyperplasias containing HCC and 75% of atypical adenomatous hyperplasias demonstrated marked fatty changes. They calculated that approximately 20% of all HCCs are perhaps multicentric in origin, and 40% of adenomatous hyperplasias undergo malignant transformation.

The clonality of atypical adenomatous hyperplasia of the liver was first demonstrated by Tsuda et al. in 1988 (98) in an HBsAg positive patient in whom a resected liver had two atypical adenomatous hyperplasias, one of which contained a small HCC. The Southern blot analysis revealed the same DNA integration pattern in both the cancer and the adenomatous hyperplastic nodule, and a different integration pattern in the other nodule. Clearly, the adenomatous hyperplasia continued clonal expansion and, after a phenotypic change (hence a genetic change) of the adenomatous cells, they transformed. There have been recent attempts to determine the clonality of nodular lesions and early HCC. Aihara et al. (99) studied the x-chromosome-linked phosphoglycerokinase gene in female patients with cirrhosis and HCC, and found that all seven cancers and 43% of regenerative nodules were monoclonal. They suggested that the monoclonal cell expansion was initiated before the nodule is established by septum formation. Kawai et al.'s approach (100) was similar and showed that all large HCCs had a monoclonal pattern, and two of four small ones each showed a mono- and a polyclonal pattern. Heavy lymphocytic infiltration modified the clonality results. Aihara and his associates (101) further studied clonality, this time in males as well, using DNA finger printing, restriction fragment length polymorphism of the x-chromosome-linked phosphoglycerokinase gene and inactivation of the gene by methylation. The results were similar to the earlier ones. All dysplastic nodules and HCC were monoclonal. In their opinion, a dysplastic nodule is not a hyperplastic but a neoplastic lesion. A French study on the clonality of macronodules in cirrhosis yielded the same conclusion (102), and the pathologists now seem to be veering towards accepting dysplastic nodules as malignant (103). The question is whether monoclonal growth is an equivalent of immortalization. Imaging finds several discrete nodules in a cirrhotic liver, and biopsy is compatible with a dysplastic nodule. Should resection or transplantation be carried out immediately? Further studies are required to elucidate other genetic markers that will identify immortalization of the monoclonally expanding cells.

Diagnosis

Alpha-fetoprotein (AFP) is the most important tumor marker for the diagnosis of HCC. However, a considerable proportion of HCCs do not produce AFP or elevate its serum level only minimally, making early diagnosis difficult with this marker alone. Des-γ-carboxy prothrombin (DCP) (104) or protein induced by vitamin K absence or antagonist II (PIVKA-II) (105) has been used as an adjuvant marker for AFP negative cases. DCP is more specific than AFP, and serum levels of AFP and DCP have no correlation, many AFP negative cases testing positive for DCP (106). The earlier test kit for DCP was less sensitive compared with the AFP test, and most small HCCs gave values within normal limits (107). The kit for PIVKA-II has recently been improved with increased sensitivity (108), and is now as useful as AFP in detecting small HCC (Fig. 3). According to Kuromatsu et al. (109), 12 of 59 patients with HCC smaller than 2 cm (20.3%) were positive, 38.3% of those having HCC of 2–3 cm tested positive, and 91.7% of HCCs larger than 3 cm were

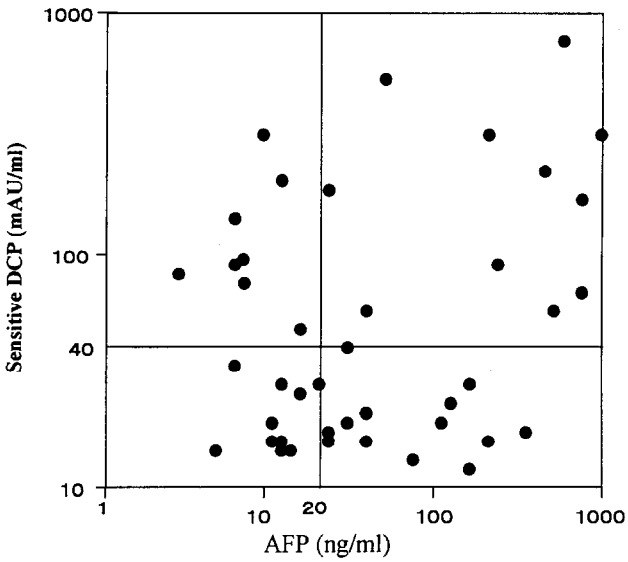

Fig. 3. Relationship between serum AFP and des-γ-carboxy prothrombin (DCP) (PIVKA-II) levels in 42 patients with HCC whose levels were below 1000 ng/ml and 1000 mAU/ml, respectively. There is no correlation. If AFP alone were measured, eight patients in the left upper compartment were missed out. (Courtesy of H. Okuda, M.D.).

positive. In the case of AFP, 35% of HCCs smaller than 3 cm give a normal value (110). Clearly, PIVKA-II has become an important marker for detection (screening) and diagnosis. AFP is often elevated to equivocally high levels (20–400 ng/ml) in serum in cirrhotic patients (111), posing another problem. AFP molecules from HCC patients are microheterogeneous (112), and Aoyagi et al. (113) demonstrated that fucosylation of the sugar chain is altered. Taketa et al. (114) found that these AFP subspecies from HCC patients have a high affinity for lectin (L) and erythroagglutinating phytohemagglutinin (P). They subsequently established an assay system for L3 and P4+P5 fractions (115). In a different approach, Aoyagi et al. developed a method to determine the degree of fucosylation (fucosylation index) which has a diagnostic sensitivity of 66% with a 93% specificity (116). Rimal et al. (117) measured L3 and P4 fractions (as percentage of total AFP) in 33 elderly people who had elevated AST and AFP levels above 20 ng/ml; five of seven individuals who had an elevated fucosylation index were found to have HCC. Sato et al. (118) tested L3 and P4+P5 fractions in 76 patients with cirrhosis having AFP levels higher than 30 ng/ml. Thirty-three were found to have HCC during the follow-up period of 35 months and L3 and/or P4+P5 fraction was elevated 3–18 months before the detection of HCC by imaging in 24 of them. In other words, elevation of these fractions is perhaps the earliest sign of HCC detectable at the moment. A more practical test kit should be developed for measuring fucosylated AFP subspecies, and a combination of total AFP, PIVKA-II and fucosylated AFP will further improve early detection/diagnosis of HCC.

The mechanism of PIVKA-II production by HCC tissue should be elucidated. Administration of vitamin K brings about a rapid reduction of elevated serum levels of PIVKA-II (106). There is no abnormality in the prothrombin gene in HCC cells producing PIVKA-II (119). Several studies have been aimed at detecting circulating and metastasizing HCC cells by demonstrating AFP mRNA in mononuclear cells in blood (120–122). These studies have shown that AFP mRNA, if detected, simply reflects an advanced stage such as a large size main tumor, hepatic vascular invasion or intrahepatic metastasis. Interestingly, patients with advanced HCC whose PIVKA-II levels are elevated all have positive AFP mRNA in the peripheral mononuclear cells (123).

Imaging modalities have greatly advanced in recent years and detection of space occupying lesions and nodules is no longer a problem. Differential diagnosis has also improved, particularly by combined use of several modalities and contrast material. One of the remarkable developments is ultrasound (US) angiography, which was initiated by Matsuda & Yabuuchi in 1986 (124). They injected CO_2 microbubbles through the catheter into the hepatic artery while using ultrasound to look at the detected lesion in the liver. Although the procedure is somewhat cumbersome, this technique was soon found to be very sensitive, capable of delineating lesions as small as 1 cm (125,126). US angiography clearly delineates a small nodule within a dysplastic nodule (nodule-in-nodule) (127), and is useful in differential diagnosis of hemangioma, liver metastases and focal nodular hyperplasia (128,129). Several attempts were then made to produce other materials that would similarly give rise to microbubbles within the blood vessels to enhance the signal within tumors (130). Schering Laboratories first succeeded in preparing an agent that consists of galactose and palmitate, and produces 2–4 μm microbubbles in blood. These bubbles are smaller than erythrocytes and pass through the lung capillaries reaching the liver, maintaining their sonic wave reflecting properties. This circumvents arterial catheterization. With color Doppler imaging, which noninvasively provides flow images for arteries and portal veins, and with the use of such contrast agents, US angiography has come to be used for a definitive and differential diagnosis (131). Dysplastic nodules show constant wave form signals, whereas overt HCC show pulsatile wave form signals (132–134).

The typical vascular patterns of HCC on US angiography are peripheral arterial supply and a homogeneous or mosaic hypervascular pattern. The detection rate for small HCCs less than 3 cm in diameter is 95% (135), better than the conventional angiography or Lipiodol CT (136). Power Doppler US, a new modality, is more sensitive than the conventional Doppler in delineating tumoral blood flow, although it does not provide directional information (137), and the use of contrast agent further augments color images (138,139). Power Doppler US is also useful in the assessment of therapeutic effects and recurrence of HCC after transcatheter embolization (137).

With helical CT imaging, CT angiography now provides an objectively evaluable delineation of HCC, precluding conventional hepatic angiography. CT arteriography (CAT) (140) followed by CT arterial portography (CTAP) (141,142) is currently the most reliable procedure for a definitive diagnosis of HCC, the former enhancing an HCC lesion and the latter producing a negatively constrasted image. By helical CT, single-level dynamic imaging is possible. With this technique, it has been found that HCC is first enhanced following contrast injection in CTA, and the contrast agent then enters the draining portal venules, which are seen as a halo around the mass. In other words, a late phase CT image gives an erroneously exaggerated size of tumor (143). The current task for the radiologist is to differentiate a benign regenerative nodule, a dysplastic nodule, and a dysplastic nodule containing an evolving early well-differentiated HCC. Such differentiation should be made whenever a small nodule is found in a cirrhotic liver based on the comparative analysis of portal and arterial blood flow which is now possible with these imaging modalities.

Treatment and prevention

The prognosis of HCC is extremely poor, even with the remarkable progress in medical science. Survival is calculated from the time of diagnosis, and early diagnosis falsely extends calculated survival; most studies have not considered it. A complete cure is only possible with liver transplantation for an early HCC without extrahepatic spread (13). This ideal therapy is rarely achieved because of the very limited availability of donor livers. Local ablation therapy first with ethanol (144–146) and then acetic acid (147), followed by microwave coagulation (148) and radiofrequency ablation (149,150), prolongs survival somewhat, but frequently new HCC develops (called recurrence) in the liver, particularly with HCV-associated cirrhosis (151). Because of these dismal experiences in treatment, more serious thought is increasingly being given to the prevention of HCC.

Prevention of HCC may be effected in three ways. The primary prevention is to avoid exposure to carcinogens and infection with a hepatitis virus. The secondary prevention is to prevent viral hepatitis from progressing to cirrhosis. The tertiary prevention is to prevent the liver that already has cirrhosis from developing HCC; this may be called "chemoprevention." The primary prevention is being achieved in some measure with vaccination programs. Prevention of transfusion-associated HBV and HCV infection has been successful in developed countries. Prevention of progression of chronic hepatitis B and C has been attempted with the use of interferon (IFN) with limited success. IFN monotherapy is being replaced by combination therapy with an antiviral agent (152). It is expected to increase the rate of resolution or prevent progression of the disease in some patients. Already, thousands of patients with chronic hepatitis B and C have been treated with IFN throughout the world. Hepatologists are keen to ascertain whether IFN treatment does indeed prevent hepatocarcinogenesis.

Ikeda et al. (153) analyzed 1643 patients with chronic hepatitis C: 1191 treated with IFN and 452 untreated. The rate of hepatocarcinogenesis was 2.1% and 4.8%, respectively, for the two groups at the end of 5 yr, and 7.6% and 12.4% in the 10th yr. The hazard of carcinogenesis was significantly lower in patients with persistently normal ALT regardless of the serum HCV RNA load, and IFN significantly lowered the rate of HCC development. Many other similar studies in Japan have almost unanimously indicated a cancer-preventing effect of IFN. However, such results have to be analyzed with caution, because HCC is also found frequently after the IFN treatment, suggesting that the patient already had undetectable HCC at the start of treatment (154).

Mazzella et al. (155) analyzed 347 patients with compensated cirrhosis, 227 treated with IFN and 120 not treated. After 32 months of follow-up, nine of 83 untreated C-cirrhosis patients developed HCC and five of 188 treated patients did. These five patients were all non-responders, and none of 74 responders developed HCC. Nishigishi et al. (156) randomized 90 patients with compensated C-cirrhosis and gave 6 MU IFN-alpha for 12–24 weeks, and followed them for 2–7 yr. HCC developed in two of the IFN group and in 19 of the non-IFN group. Oka et al. (157) carried out a similar study in 260 randomized patients with viral cirrhosis (many more C than B), one group receiving a Chinese herbal medicine "Sho-saiko-to". At the end of 5 yr of medication, 85 of 130 treated patients remained

alive without HCC and in the untreated group only 65 of 130 were free of HCC. IFN was not considered indicated for patients with cirrhosis previously, but more recent studies seem to suggest that patients with cirrhosis also benefit from IFN administration (158). Muto and his group had a different design in which patients who had had resection for HCC were randomized and subjected to chemoprevention. In a study at the National Cancer Center Hospital, postresection HCC recurrence occurred in 54% of the patients within 15 months (159). They gave polyprenoic acid, an acyclic retinoid which inhibits chemically induced hepatocarcinogenesis in rats and spontaneous HCC in mice, to 44 resected patients and a placebo to 45 resected controls. After a follow-up of 5 yr, a significant difference was found with a lower rate of secondary HCC emergence in the treatment group. The study was further extended in follow-up and the difference was still clearly demonstrable (160). They postulate that this acyclic retinoid induced clonal deletion in premalignant and latent malignant cells.

The real and practical question for the hepatologist who sees patients with chronic viral disease is whether those who already have cirrhosis should be given IFN plus an antiviral agent or carotenoid as a means of chemoprevention. We need more chemopreventive agents from which an appropriate one could be chosen for each patient who is potentially at risk of developing HCC. This author is of the opinion that the prospect for further improvement or progress in therapy for HCC is not very bright, and more serious thought and effort need be directed toward prevention.

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