Precursor Lesions for Liver Cancer in Humans

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Summary

Our knowledge of the cellular changes that lead to liver cell carcinoma in humans is limited by proper and necessary ethical restriction on clinical research. We know rather more about risk factors, the most important of which is cirrhosis. It seems that both the causative agent and the time of duration of the cirrhotic process are relevant to the magnitude of this risk. According to present knowledge, α1-antitrypsin deficiency, alcoholism, naturally occurring carcinogens, drugs, and the hepatitis B virus seem to carry the greatest risk of cancer developing in a cirrhotic patient. Cirrhosis, however, is not an essential prerequisite, and some or possibly all of these agents can also induce cancer without cirrhosis. Bile duct carcinoma commonly follows infestation with liver flukes. Cirrhosis is usually absent but duct epithelial hyperplasia is present prior to the development of cancer. Many cellular changes have been observed in patients and among populations considered to be at risk from liver cancer. Of these, liver cell dysplasia is the most striking and studies of its prevalence, natural history, and association with cirrhosis suggest that it is a precancerous change.

Introduction

There is a considerable body of evidence, based on experimental animal models, to suggest that carcinogenesis is not a sudden, single-step event but a gradual, multistep process. It begins with an "initiation" phase which may be quite short, a matter of hours or days. This is then followed by a much longer period of "promotion," during which neoplastic development takes place through the successive emergence of new cell populations, each more abnormal than its predecessor (13). Much has been learned in recent years about the nature and manifestations of these changes but infinitely more is yet to come.

Experimental pathology applies sophisticated scientific methods to situations in which a proven or putative carcinogen, its dose, route of administration, and most secondary factors are known and where the sequence of events can be studied in a predetermined manner. Research on humans, whether by clinical, laboratory, or epidemiological means, is often haphazard by comparison. It depends on the analysis of data that, in the absence of any certain knowledge of the forces at work or of their strength and duration, can only be collections of chance findings or inspired guesswork. Ethical considerations are paramount and preclude any investigative procedure unless it be for the benefit of the patient. Thus we rarely have the opportunity to study sequential changes in humans and only too often we have nothing but the end result to contemplate.

It is remarkable, nevertheless, that so much has been learned by such limited means. We may know comparatively little about the cellular events that precede neoplasia in humans but we do know a great deal about the circumstances that surround them.

Cirrhosis

Putative precursor lesions in human liver cancer include most forms of chronic liver disease, the common morphological expression of which is cirrhosis. There are many aspects to the relationship between these 2 conditions. These have been exhaustively if inconclusively studied (5, 10, 18), but a basic pattern is beginning to emerge.

Liver cell carcinoma in humans shows a remarkable geographical variation, with the highest incidence in sub-Saharan Africa and Southeast Asia. We have no firm evidence that cirrhosis is any commoner in these areas and it may even be less so. In parts of Africa, such as Mozambique, up to 40% of liver cell carcinomas develop in noncirrhotic livers. Cirrhosis cannot, therefore, be an essential prerequisite to cancer, as indeed it is not in the experimental animal. In North America and Western Europe, however, the recent, gradual rise in the incidence of liver cell carcinoma has been attributed to a similar increase in the prevalence of cirrhosis. This is partly due to an increase in the number of patients with cirrhosis and partly to their longer survival with better methods of treatment (25, 30). The morphological pattern of cirrhosis also differs in high- and low-incidence areas. In Africa and Southeast Asia it is commonly macronodular, i.e., Gall's posthepatitic or postnecrotic type, whereas in North America and Europe it is generally micronodular, i.e., Gall's nutritional, fatty, or alcoholic type (18). The risk of cancer is much greater in macronodular types of cirrhosis (14). Such differences in morphology may reflect differences in etiology, toxic/infective in the tropics and alcoholic in Western countries (3). They also depend on the duration of the process. Several studies have shown that cirrhosis, of whatever etiology, tends to progress towards a macronodular pattern and that this is associated with an increased risk of cancer (24).

Bile duct carcinoma in humans takes 2nd place to liver cell carcinoma. Its incidence is lower and is geographically more uniform, with the exception of parts of the Far East where both types of tumor are common (15). Bile duct carcinoma is not associated with cirrhosis but it is associ-
ated with infestation of the biliary tree by *Clonorchis sinensis* and other liver flukes.

A further type of cancer of the liver is hemangiosarcoma, which has, until recently, been considered excessively rare in humans and a mere curiosity. We may be witnessing the beginning of an epidemic of this disease due to industrial exposure to vinyl chloride monomer (34). Hemangiosarcoma is not associated with cirrhosis but it is preceded by a form of chronic liver disease that mainly affects the blood vessels and their endothelial lining.

This brief survey of the background to the main types of liver cancer in humans helps to highlight the main problem, which is that of liver cell carcinoma and its relationship to the causative agents of chronic liver disease in general.

**Miscellaneous Factors and Lesions**

The evidence for linking a particular factor or lesion with the subsequent development of liver cell carcinoma is often tenuous and rarely conclusive.

**Age, Sex, and Genetics.** In areas where liver cell carcinoma is common, it affects people in youth and early adulthood. It is widely accepted that the early onset of the disease is due to the intensity of exposure to environmental carcinogens rather than to any genetically determined susceptibility (18). The best type of evidence we have for this is the fact, shown by several studies on different populations, that migrants from high- to low-incidence areas quickly adopt the low rates of their new environment. Males are more susceptible to liver cancer in all areas, and we have no adequate explanation for this.

The recent discovery of \( \alpha_1 \)-antitrypsin deficiency and its relationship to cirrhosis and, possibly, to liver cancer is of interest. The enzyme is produced by the liver under the influence of at least 23 different codominant alleles. Individuals of the PiZZ genotype show the lowest blood levels and are liable to the early development of chronic lung and liver disease. Recently, an unusually high incidence of liver cell carcinoma has also been noted (12). The deficiency state is characterized morphologically by the presence of periodic acid-Schiff-positive material in hepatocytes that has been shown to consist of an incomplete form of \( \alpha_1 \)-antitrypsin. The defect probably lies in the final assembly for release by the Golgi apparatus of the liver cell, but how this leads to cirrhosis and cancer is unclear.

The progressive changes involved in neoplastic development must be subject to a number of selective and inhibitory mechanisms. One of these at least, the immune response, is subject to genetic control. Histocompatibility or HL-A loci have been shown to be linked to various forms of human disease including chronic liver disease (23). Whether individuals with specific HL-A genotypes are more prone to cancer is not known at present and needs to be investigated.

**Alcohol and Malnutrition.** Liver disease of the alcoholic is probably due to alcohol itself rather than to any coexisting malnutrition (33), but the matter is far from settled (17). Alterations of circulating immunoglobulins, lymphocyte responsiveness and T- and B-cell functions are also found (6). The changes found range from fatty liver to alcoholic hepatitis and cirrhosis, sometimes with siderosis. These changes are sequential and each is associated with characteristic clinical, laboratory, and histological findings. Individual liver cell damage and necrosis, progressive fibrosis, and regeneration are evident throughout but cancer rarely arises during this active phase. Lee (24) has shown that it is the reformed alcoholic who has survived his habit who is at greatest risk. In such individuals cirrhosis is often of the macronodular pattern which, in any situation, is the most likely to be precancerous (14). It is doubtful whether siderosis has much role to play.

**Parasites.** There is no evidence that parasites, such as plasmodia, schistosomes, and amebae, can cause liver cancer in humans. In parts of the Far East, however, where infestation with liver flukes is common, both liver cell and bile duct carcinomas are highly prevalent. Of the 2, only the latter has been linked with flukes (*Clonorchis sinensis*, *Opisthorchis viverrini*, and others). The presence of these organisms is associated with proliferation, budding, and increasing disorganization of intrahepatic bile ducts (Fig. 1). Mucus production is excessive but the mucin produced is the same as that normally produced in the biliary tree. Various enzymes have been identified in the metabolic products of the flukes but no carcinogenic substances have yet been found. The subject has been recently reviewed by Gibson and Chan (15).

**Naturally Occurring Carcinogens.** The term "naturally occurring carcinogens" refers to a vast array of substances, many of which have been found in foodstuffs, native remedies, and the like. Nearly all are putative only and few have been studied in any detail.

Pyrolizidine alkaloids affect mainly the liver, where they produce somewhat variable results according to species. In rodents a wide variety of changes is produced, including fibrosis, bile duct proliferation, nodular parenchymal hyperplasia, and megalocytosis (9). In humans the main changes are those of venoocclusive disease, in which narrowing and obliteration of hepatic vein radicles predominate (8). In the West Indies, where ingestion of pyrolizidine alkaloids is common in the form of "bush teas," liver cancer is not unduly common.

Nitrosamines are among the most powerful experimental liver carcinogens known (26). There is no reason to believe that humans are not susceptible. There is some published evidence of the occurrence of nitrosamines in foodstuffs and of their formation from nitrates and amines inside the body. This is suggestive evidence that they may cause disease in humans.

The chemistry, biological effects, and carcinogenicity of mycotoxins have been extensively studied (38). Many animal species are susceptible, and exposure of humans to these substances undoubtedly occurs. Surveys in Africa and Southeast Asia, where primary liver cancer is common, show that up to 65% of groundnuts and 30% of rice sampled are contaminated with aflatoxins, a group of compounds produced by the mold *Aspergillus flavus*. An exceptionally well designed and executed study in Kenya showed a close correlation among climatic conditions, aflatoxin consumption, and the incidence of liver cancer (29). The
pathological changes induced in the liver by aflatoxins have been reviewed by Newberne and Butler (28). They range from acute parenchymal necrosis to cirrhosis with cytological abnormalities and liver cell carcinoma.

**Drugs and Chemicals.** A wide variety of drugs and chemicals is known to cause acute liver necrosis, hepatitis, cholestasis, fibrosis, and cirrhosis. The danger of a carcinogenic potential is always present, and we are perhaps fortunate that few examples have emerged so far. Thorotrast, a radiological contrast material (20), arsenic (32), and more recently vinyl chloride monomer (34) have been found to be capable of producing angiosarcoma of the liver. Even more worrying is the possibility that liver tumors may result from the use of gonadal steroids. The use of oral contraceptive agents has been linked to liver cell adenomas (4) and that of androgenic/anabolic steroids to liver cell carcinoma (22). A stream of case reports continues to swell the literature, but really convincing evidence will have to come from well-controlled and large-scale studies. We know little about premalignant changes in these cases, although we do know a great deal about the effect of steroids on the liver in general (11). These include interference with the bile secretory function of the cell, hypertrophy of the endoplasmic reticulum, and giant mitochondria, as well as some vascular effects, namely thrombosis of hepatic veins and peliosis hepatis.

**Hepatitis.** Pathologists working in the tropical countries of Africa and Southeast Asia have long been impressed by the large amount of chronic inflammatory liver disease prevalent in those areas (21). The appearances range from minor liver cell abnormalities, Kupffer cell hyperplasia, and portal tract inflammation to chronic hepatitis of variable severity and established cirrhosis with a marked inflammatory component. Much earlier writing has centered on the question of how much of this could be attributed to the many infective and toxic agents widely prevalent in the tropics and how much to specific causes such as viral hepatitis. The general assumption has been that sporadic viral hepatitis was probably the major factor, but in the absence of a marker for this disease the problem could not be settled for certain.

The situation has changed dramatically since the discovery of the hepatitis B antigen, which is now widely accepted to be a marker for hepatitis B infection. This form of hepatitis accounts for most sporadic cases, whereas epidemics are mainly caused by hepatitis A. Recent advances in this field have been the subject of several reviews (7, 36, 39). At the moment it seems that hepatitis B rather than hepatitis A is responsible for long-term liver damage.

The definitive account of chronic hepatitis B infection will not be written for many years yet, not until long-term follow-up studies have provided the necessary data. In the meanwhile we have to turn our attention to those with existing liver disease.

Acute viral hepatitis B was soon linked to the antigen but the association with chronic liver disease was, at first, slow to appear. The 1st controlled studies were reported from Uganda (27, 37) and from Taiwan (35). They were rapidly followed by numerous further reports of a significant association between the presence of the hepatitis B antigen and chronic liver disease, namely cirrhosis and liver cell carcinoma. This association is particularly strong in the tropics. The current situation is reviewed by Prince (31). Such an association, however, need not necessarily indicate a cause-to-effect relationship, and a number of alternative explanations has been proposed, including genetic susceptibility to hepatitis B infection and impaired immunity or tolerance. The sort of evidence that is needed must show that infection precedes the development of the tumor, that virus-specific antigens are present in it, and that the virus can transform cells in culture or induce cancer in the livers of experimental animals. Finally, immunization against hepatitis B should reduce the incidence of liver cell carcinoma in those parts of the world where the population is known to be at risk. None of these conditions has been fully met so far but there are other suggestive items of evidence for a direct oncogenic role. The hepatitis B antigen contains RNA-dependent DNA polymerase, which is a constant property of RNA tumor viruses in animals (19). The antigen has also been found to be associated with liver cell dysplasia.

**Liver Cell Dysplasia**

The term refers to cellular enlargement, nuclear pleomorphism, and multinucleation of liver cells occurring in groups or occupying whole cirrhotic nodules (Figs. 2 to 4). Liver cell dysplasia is certainly abnormal and probably precancerous. This is based on a large scale study of its prevalence, natural history, and association with particular types of cirrhosis for details of which the reader is referred to the original publications (1–3). In brief, dysplasia was found in only 2 of 200 (1%) patients in normal livers, in 3 of 43 (6.9%) of patients with normal livers bearing primary liver cell carcinoma, 35 of 175 (20.3%) patients with cirrhosis, and 80 of 124 (64.5%) of patients with cirrhosis and primary liver cell carcinoma. No association was found between liver cell dysplasia and bile duct carcinoma. Cirrhotic patients without dysplasia were an average of 10 years younger than those with dysplasia, and the latter were an average of 6 years younger than those with cirrhosis and carcinoma. Liver cell dysplasia occurred more frequently in males than in females. It was found in all but 1 instance in macronodular or mixed forms of cirrhosis only. Similar changes had previously been described under a variety of terms in many parts of the world but particularly in the tropics, where a liver cell carcinoma is common.

A strong relationship was found between liver cell dysplasia and the presence of the hepatitis B antigen. From this it was speculated that dysplastic liver cells perhaps represented an example of suppression of cancer by cell fusion in the manner suggested by Harris et al. (16). They showed that the malignant potential of cells from 3 types of tumors, 1 of which had been induced by a virus, could be suppressed when fused with certain nonmalignant cells. The hybrids resulting from such fusions produced segregants in which a loss of chromosomes was associated with reversion to malignant behavior after a variable period of time. It is possible at least that the hepatitis B virus induces malignant change in liver cells, which then fuse with normal liver cells to form large, abnormal, dysplastic
cells. Cancer is thereby suppressed and in some instances this may be permanent. In others, the hybrid gradually escapes through loss of chromosomal material and malignant behavior is then resumed.

References

Fig. 1. A large bile duct in the liver with a gravid female clonorchis fluke lying free in the lumen. The epithelial lining of the duct shows marked adenomatous proliferation. H & E, × 65.

Fig. 2. Liver cell dysplasia: large cells with grossly pleomorphic, hyperchromatic nuclei (top left) contrasted with normal liver cells (bottom right). H & E, × 250.

Fig. 3. A well defined group of dysplastic liver cells among normal cells. H & E, × 100.

Fig. 4. A whole cirrhotic nodule shows dysplasia (right). H & E, × 100.
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