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REVIEW

Hepatocellular carcinoma in African Blacks: Recent progress in etiology and pathogenesis

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Abstract

Occult hepatitis B virus (HBV) infection was shown to be present in 75% of Black Africans with hepatocellular carcinoma (HCC) in whom the tumor was hitherto not thought to be caused by chronic HBV infection. The association between chronic HBV infection and the development of the tumor is thus even closer than was originally thought. HBV viral load was found to be significantly higher in patients with HCC than in Black African controls. As in other populations, HBV e antigen-positive patients with hepatocellular carcinoma had significantly higher viral loads than patients negative for this antigen. The significance of this finding is discussed. The risk for HCC development with genotype A of HBV, the predominant genotype in African isolates, has not been investigated. Genotype A was shown to be 4.5 times more likely than other genotypes to cause HCC in Black Africans, and tumours occurred at a significantly younger age. Increasing numbers of patients with human immunodeficiency virus (HIV) and HBV co-infection are being reported to develop HCC. A preliminary case/control comparison supports the belief that HIV co-infection enhances the hepatocarcinogenic potential of HBV. A study from The Gambia provides the first evidence that dietary exposure to aflatoxin B_1 may cause cirrhosis and that this may play a contributory role in the pathogenesis of aflatoxin-induced HCC. An animal model has provided experimental support for the clinical evidence that dietary iron overload in the African is directly hepatocarcinogenic, in addition to causing the tumor indirectly through the development of cirrhosis.

Key words: Hepatocellular carcinoma; Black Africans; Occult hepatitis B; Virus infection; Hepatitis B viral loads; Hepatitis B virus genotype A; Aflatoxin; Dietary iron overload

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INTRODUCTION

Sub-Saharan Africa is one of three geographical regions where hepatocellular carcinoma (HCC) occurs very commonly. The high incidence of the tumor is confined to the Black population of the sub-continent. Published incidences of HCC in sub-Saharan Africa underestimate its true incidence because in many instances the tumor is

either not definitively diagnosed or is not recorded in a cancer registry.

A number of differences exist between HCC that occurs in sub-Saharan Africa and that seen in other parts of the world. The tumor generally presents at a younger age in African Blacks than it does in the populations of industrialized countries, and the male preponderance is more striking. Rural and rural-born Blacks have a higher incidence of the tumor than do urban-born Blacks. Although the prognosis of HCC is poor in all geographical regions, it is especially grave in African Blacks, in whom the annual fatality ratio of the tumor is 0.97. The fibrolamellar variant of HCC is rare in African Blacks.

Chronic hepatitis B virus (HBV) infection is the predominant cause of HCC in sub-Saharan Blacks, accounting for the great majority of the cases. The infection is almost always acquired in early childhood, usually by horizontal transmission of the virus. Recently infected and hence highly infectious young siblings or playmates are most often the source of the infection. Perinatal transmission of the virus plays a lesser but still important role. Rural and rural-born children and adults have a higher incidence of chronic HBV infection than do their urban counterparts. Chronic hepatitis C virus (HCV) infection is a less common cause of HCC in sub-Saharan Africa. Patients with HCV-induced tumors are generally about two decades older than those caused by HBV and the gender and rural-urban differences are less obvious. HCV and HBV act synergistically in causing HCC in African Blacks. Another important risk factor for the tumor in sub-Saharan Africa is prolonged heavy dietary exposure to the fungal toxin, aflatoxin B_1 (AFB_1), and there is a strong synergistic interaction between this toxin and HBV in causing the tumor. Heavy exposure to AFB_1 is virtually confined to rural areas. More recently, another important cause of HCC in African Blacks has been recognized. Originally referred to as Bantu visceral siderosis, the term dietary iron overload in the African is now preferred. Consumption of large volumes of a home-brewed traditional beer that has a high iron content is the cause of the condition, although a genetic predisposition may play a role. Over time, the resulting hepatic iron overload may be complicated by HCC development.

Some aspects of the recent progress in understanding the etiology and pathogenesis of HCC in African Blacks are summarised in this review.

OCCULT HBV INFECTION AND HCC

During recent years occult HBV infection has emerged as an important and challenging entity. It is defined as the presence of HBV DNA in low concentration in liver tissue of individuals whose serum persistently tests negative for HBV surface antigen with conventional serological assays, but in whom HBV DNA is usually but not invariably detectable in their serum using highly sensitive molecular assays[1]. When detectable in the serum, the amount of HBV DNA is usually very low (< 200 IU/mL). On the basis of the profile of HBV antibodies in serum, occult HBV infection may be divisible into seropositive (anti-HBc and/or anti-HBs positive) and seronegative (anti-HBc and anti-HBs negative)[1].

Because HBV infection is endemic in sub-Saharan Africa and chronic overt infection with this virus is the major cause of HCC in African Blacks, it is necessary to know the prevalence of occult HBV infection in this population and to ascertain if it plays a role in the aetiology of the tumor. Although early studies had hinted that low concentrations of HBV DNA in the serum of African Blacks with HCC may be undetected by conventional serological tests[2-4], occult HBV infection was first documented in this population in 2001[5]. The few studies published subsequently have indicated that occult HBV infection occurs in an additional 0.6% to 1.7% of sub-Saharan Blacks[6-8] over and above the average prevalence of overt HBV infection of 8% to 10%[9,10].

Because human immunodeficiency virus (HIV) is also endemic in sub-Saharan Africa and co-infection between this virus and HBV occurs in as many as 17% of the Black population[11,12], the prevalence of occult HBV infection in HBV/HIV co-infected individuals has recently been investigated. In the first study, a retrospective analysis of sera stored in a routine diagnostic laboratory, 5 of 15 HIV-positive individuals had occult HBV infection compared with none of 31 HIV-negative individuals[13]. In the second study, 4.8% of a large cohort of HIV-positive individuals had overt HBV infection and a further 7.4% had occult infection[14].

Sixty to 70% of African Blacks with HCC are overtly infected with HBV at the time the tumor is diagnosed[9,10]. The relative risk of HBV carriers developing HCC ranges from 9 to 23.3[15-17], with young Blacks carriers being at greater risk of tumor formation than their older counterparts[18]. The occurrence of occult HBV infection in HCC in southern African Blacks has recently been investigated[19]. A sensitive polymerase chain reaction (PCR) assay was used to amplify the DNA of the surface and precore/core genes from the serum of patients with HCC that was known to be negative for HBsAg but positive for HBV antibodies. Positive bands were confirmed by nucleotide sequencing. Surface gene HBV DNA was detected in a single PCR assay in 48.4% of the patients. Because of the known variability of the results of PCR assay at low concentrations of HBV DNA[20,21], a second assay was done, which increased the positivity rate to 57.7%. Two further assays increased the rate to 75.7%. A less sensitive PCR assay, of the precore/core region, yielded corresponding positive results in 23.7%, 32.2%, and 52% of the patients. The study concluded that seropositive occult HBV infection was present in the majority of African Black patients with HCC without overt HBV infection, and that the causal association between chronic HBV infection and HCC might therefore be even closer than was previously believed.

This study did not address the question of whether or not occult HBV infection *per se* causes malignant transformation of hepatocytes. In early studies of African Blacks with HCC the presence of HBV DNA as well as HBV RNA, indicating viral transcription, was demonstrated in the tumor tissue of some patients whose serum was negative for HBsAg[3,4]. With hindsight, this finding suggests that occult HBV infection might play a role in the pathogenesis of HCC in African Blacks. However, further investigation of the molecular genesis of the tumor and prospective molecular epidemiological studies in patients with occult HBV infection will be required to prove or disprove such a role[1].

HBV LOADS IN HCC

A number of host-specific variables and viral characteristics of chronic HBV infection are known to influence the risk of HBV-induced HCC. These include HBV e antigen (HBeAg) status, the presence of chronic necroinflammatory hepatic disease (particularly cirrhosis), gender and age, as well as viral load and genotypes and possibly subgenotypes of the virus. The availability during recent years of laboratory techniques that accurately quantitate the number of HBV particles in the serum of infected individuals has provided the opportunity to evaluate more fully the role of viral load in the pathogenesis of HCC. Long-term follow-up studies of HBV carriers in Taiwan, China, and Japan have shown that past high viral loads correlate with an increased risk of progression to malignant transformation[22-30]. This risk is influenced by a number of variables, including HBeAg status[30], genotype[31], male gender[30], and possibly age[32].

HBV-induced HCC in African Blacks differs from that in other populations in certain ways and these might either influence the viral load or be the result of differences in the viral load. To date, a single study of past HBV viral loads and involving only 14 Senegalese subjects has been reported in African Black patients with HCC[25]. The difficulty in performing meaningful long-term follow-up studies in southern African Blacks with chronic HBV infection precludes the possibility of directly determining the role played by past viral load in predicting the risk of malignant transformation in these individuals. In a recent attempt to partly circumvent this obstacle, viral loads measured by real-time PCR assay in a cohort of southern African Black male patients with HBV-induced HCC were compared with those in a cohort of asymptomatic Black male carriers of HBV[33]. The mean value of the viral loads in the HCC patients was appreciably higher than that in the asymptomatic controls. Of the HCC patients, 62% had viral loads greater than 1 × 105 copies per mL and 87% loads greater than 1 × 104 copies per mL, compared with 15% and 49.6%, respectively, of the carriers. Therefore, almost all of the HCC patients and one-half of the carriers had high viral loads.

As in other populations studied previously, HBeAg-positive HCC patients had significantly higher viral loads than HBeAg-negative patients. Of the HBeAg-positive patients, 84% had copy numbers per mL of greater than 1 × 105 and 96% greater than 1 × 104, compared with 57% and 85%, respectively, in the HBeAg-negative patients. Thus, although the values were lower in the HBeAg-negative patients, the majority of these patients had high viral loads. This finding may be relevant to the observation that African Black carriers of HBV seroconvert from HBeAg-positivity to negativity at a far earlier age than occurs in other populations (only 5% are positive in adulthood compared with 40% or more in Chinese carriers[34]) and yet are at high risk of developing HCC. Support for an association between HBeAg-negativity and the development of HCC in African Blacks, and perhaps other populations, is provided by the observations that a proportion of anti-HBe-positive European carriers retain high serum levels of HBV DNA[35,36], and that in a small series of Taiwanese patients HBeAg-negativity was shown to correlate with high viral loads and an increased risk of HCC, albeit a lesser risk than that associated with HBeAg-positivity[27]. The few Senegalese patients in whom viral loads were measured in the earlier study were equally likely to be HBeAg-positive and anti-HBe-positive[25] although, in a multivariate analysis, HBeAg-positivity alone carried an increased risk of HCC development. This finding is similar to the experience in Taiwanese patients in whom high viral loads in HBeAg-positive but not in anti-HBe-positive patients carried an increased risk of HCC development[31].

No differences in viral loads were found in relation to age, gender, or genotype in the African Black HCC patients. African Black and Chinese patients with HCC are often younger than their counterparts in industrialized countries. Little is known about the role of viral load in HBV-induced HCC occurring at a young age[37], although one recent study from Taiwan reported that HCC patients younger than 40 years of age had lower HBV DNA levels than older patients[33]. Despite the fact that male gender is one of the factors associated with an increased risk of HCC, studies on viral loads have either been comprised of men only or have not commented on any differences between the genders with respect to viral loads. Correlation between genotypes and viral loads has been performed in a single study only. Genotype C, particularly Ce, was shown in Taiwanese and Chinese patients to carry a greater risk of HCC development and to be associated with higher viral loads than genotype B[29,31,38].

Long-term follow-up studies are needed in Black African populations to further assess the role of past HBV viral loads as a risk factor for the subsequent development of HCC. A long-term study of chronic carriers of HBV is well advanced in The Gambia and should provide the required information in due course.

INCREASED HEPATOCARCINOGENIC POTENTIAL OF HBV GENOTYPE A

HBV is composed of 8 genotypes (A to H), each differing

from the others by a total nucleotide diversity of at least 8%[39-42]. Subgenotypes, each differing from the others by a total nucleotide diversity of at least 4%[42,43], have been described for 6 of the genotypes. The genotypes have different geographical distributions[39-42] and are proving to be an invaluable tool in tracing the molecular evolution and spread of HBV. Functional differences between the genotypes at the translational level may influence the course and severity of the disease caused and its response to treatment[42,44-46]. In regions where HBV is endemic and HBV-induced HCC common, recent evidence for differing risks of tumor development between the genotypes has emerged[47-49]. The early studies in this regard concerned patients infected with genotypes B, C, or D.

Genotype A predominates in southern Africa, constituting approximately 70% of isolates, with genotype D accounting for most of the remainder (approximately 20%)[42,50-52]. Genotype A comprises 3 subgenotypes, A1, A2, and A3[42,50-52], with A1 predominating in southern Africa. No studies of the hepatocarcinogenic potential of genotype A have been published until recently, and in the only study of genotype D Thakur and co-workers concluded that this genotype may predict the occurrence of HCC in young HBV carriers[49].

In a recent study of the hepatocarcinogenic potential of genotype A[52], the genotype and subgenotype patterns in unselected southern African Blacks with HBV-induced HCC were compared with those in apparently well chronic carriers of the virus matched for sex and approximately for age. The genotyping method of Lindh *et al*[53] was used to discriminate between the genotypes and an 'in-house' restriction fragment length polymorphism pattern assay between the subgenotypes[52]. Genotype A was shown to be present in 86.5% and genotype D in 8.1% of the HCC patients compared with genotype A in 68.5%, genotype D in 23.4% and genotype E in 2.7% of the carriers. Based on these findings, the relative risk for developing HCC in the patients with genotype A (compared with those with genotypes other than A) was calculated to be 4.5 (95% confidence limits 1.86; 10.9). Not only were African Blacks infected with genotype A at greater risk of developing HCC than those infected with other genotypes (mainly genotype D), but they also did so at a significantly younger age (mean age 39.0 ± 1.4 compared with 45.4 ± 4.2 yr). Subgenotype A1 was present in all of the HCC patients infected with genotype A and also in all but one of the controls with this genotype.

Although evidence for the hepatocarcinogenic potential of genotype A in African Blacks is now available, further studies on the hepatocarcinogenic potential of the A subgenotypes are needed.

INCREASING OCCURRENCE OF HCC IN HIV/ACQUIRED IMMUNODEFICIENCY SYNDROME (ADIS) PATIENTS CO-INFECTED WITH A HEPATITIS VIRUS

Chronic infection with HIV alone does not cause HCC in humans[54,55]. HIV is transmitted in similar ways to HBV and HCV, and co-infection between HIV and HBV or HCV is common in clinical practice[56,57]. Since the introduction of highly active antiretroviral therapy (HAART) for HIV/AIDS, increasing numbers of patients co-infected with HIV and HCV or HBV have been reported to develop HCC[54,58-64]. One plausible explanation for this observation is that the considerably longer survival of patients now treated with HAART compared with those who earlier had received largely ineffective anti-retroviral drugs allows sufficient time for hepatitis virus-induced HCC to develop[64]. Another possible explanation is that the immune deficiency caused by HIV infection results in higher HBV viral loads, which are known to increase the risk of malignant transformation of hepatocytes (*see section on HBV viral loads and HCC*). Alternatively, co-infection with HIV may directly enhance the hepatocarcinogenic potential of the hepatitis viruses. In this regard, the observation in transgenic mice that HIV *tat* protein expressed constitutively in the liver enhances the effect of a number of carcinogens[65] and results, after a long latency, in a high incidence of HCC[66] may be relevant. Moreover, the *tat*-binding protein interacts with HBV × gene, which is thought to play an important role in the pathogenesis of HBV-induced HCC, in such a way as to regulate HBV transcription[67], and the HBV × protein induces HIV-1 replication and transcription in synergy with *tat* protein and T-cell activation signals[68].

A retrospective analysis of the occurrence of HCC in HIV/AIDS patients with hepatitis virus co-infection before the HAART era would be of limited use because of the brief survival of these patients at that time, and a prospective study of the development of the tumor in HIV/AIDS patients with dual infection receiving or not receiving HAART is ethically unacceptable. A case/control comparison of the occurrence of HIV in patients with hepatitis virus-induced HCC with that in matched asymptomatic carriers of hepatitis virus might, however, provide some information in this regard.

In a recent such study the prevalence of HIV co-infection in a cohort of southern African Black patients with HBV-induced HCC from the time before HAART became available to these patients was compared with that in a cohort of age- and sex-matched apparently healthy Black carriers of HBV from the same time period[69]. HIV-1/HIV-2 antibodies were found to be present significantly more often in the HCC patients than in the controls. The prevalence of HIV in the carriers was in keeping with prevalences recorded in the South African Black population at that time[70]. This observation is compatible with the belief that HIV co-infection enhances the hepatocarcinogenic potential of HBV. However, relatively few HCC patients and controls were included in this study, and further and more comprehensive investigation into a possible hepatocarcinogenic interaction between these two viruses is needed.

Because the molecular genesis of HBV-induced and

HCV-induced HCC differs[71] an interaction between HBV and HIV in the development of HCC does not mean necessarily mean that a similar interaction would occur with HCV-induced HCC.

AFB1 EXPOSURE AND HCC

Cirrhosis, whatever its cause, is a premalignant condition. In most parts of the world 80% to 90% of HCCs occur in patients with underlying cirrhosis. The proportion of African Black patients in whom the HCC arises in a cirrhotic liver is generally less than that in other populations but is still high. Of the major risk factors for HCC in sub-Saharan Africa, HBV, HCV, and dietary iron overload are all capable of causing cirrhosis. Short-term heavy dietary exposure to AFB_1, the other major causal association of the tumor in sub-Saharan Africa, causes severe acute hepatic necrosis (acute aflatoxicosis), which is often complicated by acute hepatic failure and a fatal outcome. However, long-term exposure to the fungal toxin has not hitherto been thought to cause chronic necroinflammatory hepatic disease in the form of chronic hepatitis or cirrhosis. Cirrhosis has therefore not been incriminated in contributing to the pathogenesis of AFB_1-induced HCC.

A recent study from The Gambia provides the first evidence that this may not be true. Kuniholm[72] and co-workers used an ultrasound-based method to diagnose the presence of cirrhosis in 97 Black Africans. A score of at least 7 out of a possible 11 points on the ultrasound-based scale was the criterion for the diagnosis of cirrhosis. This method has a 77.8% sensitivity and 92.5% specificity in comparison with liver biopsy in identifying cirrhosis in HBV-infected patients[73,74]. Three hundred and ninety seven individuals with no evidence of liver disease and a normal serum AFB_1 concentration served as controls. Long-term exposure to AFB_1 was assessed in the patients with cirrhosis and the controls on the basis of two observations: A history of lifetime groundnut (peanut) intake or the finding in the serum of a genetic marker of heavy exposure to AFB_1, the 249^{ser} p53 mutation. An increased relative risk of cirrhosis development of 2.8 (95% confidence interval 1.1-7.7) was calculated using a history of life-time dietary intake of groundnuts as the criterion for significant exposure, and of 3.8 (95% confidence interval 1.5-9.6) using the finding of the 249^{ser} p53 mutation in serum as the criterion, allowing for the possible confounding effect of HBV and HCV infection in each instance. A synergistic interaction between AFB_1 intake and HBV infection in the development of cirrhosis was also shown. If confirmed, this finding will provide the first evidence that cirrhosis may play a contributory role in the pathogenesis of AFB_1-induced HCC.

Because contamination of staple crops by *Aspergillus* species occurs both during growth of the crops and as a result of their improper storage, attempts at primary prevention of AFB_1-induced HCC must be directed to minimizing both sources of fungal contamination.

The likelihood of contamination during storage is increased by excessive moisture and any form of damage to the crops.

The success of postharvest intervention measures has recently been demonstrated in a study carried out on subsistence farms in the lower Kindia region of Guinea, West Africa[75]. Farms from 20 villages were included, 10 of which implemented a package of postharvest measures to restrict AFB_1 contamination of the groundnut crop, and 10 followed the usual postharvest practices. Intervention measures included hand sorting of the groundnuts to identify and discard those that were visibly mouldy or had damaged shells; sun drying of the groundnuts on natural fibre mats rather than directly on the earth to lessen contact with damp soil and to facilitate gathering in the event of unexpected rain; complete sun-drying of groundnuts confirmed by shaking the kernels to listen for free movement of dried nuts; storage in natural fibre bags rather than plastic bags (which increase humidity); storing the groundnuts on wooden pallets rather than the floor and in well-ventilated, rain-proof storage facilities to reduce humidity; sprinkling of insecticides on the floor of the storage facilities to minimise insects, which produce humidity via metabolic activity, and spread the fungal spores.

The concentration of AFB_1-albumin adducts in the serum of 600 people was measured immediately after harvest and at 3 and 5 mo postharvest. In the control villages the adduct concentration increased from a mean of 5.5 pg/mg postharvest to 18.7 pg/mg 5 mo later. By contrast, in the intervention villages after 5 mo of storage the adduct concentration (7.2 pg/mg) was much the same as that immediately postharvest (8.0 pg/mg). At 5 mo 2% of the people in the control villages had non-detectable adduct concentrations compared with 20% of those in the intervention villages.

Thus, simple, low-technology, and inexpensive practices can result in a striking decrease in exposure to AFB_1. Provision of the means to improve storage facilities and training of subsistence farmers in their use will be required for these interventions to be successful on a wide scale in resource-poor countries.

DIETARY IRON OVERLOAD IN THE AFRICAN AS A CAUSE OF HCC

Dietary iron overload occurs in several sub-Saharan African countries, where it may affect as many as 15% of Black adult males[76]. The liver is the main organ of iron storage and high hepatic iron concentrations, comparable with those in the genetically determined iron storage disease, Hemochromatosis gene (HFE) hereditary hemochromatosis, result from the consumption over time of large volumes of home-brewed beer rich in iron. The high iron content results from the preparation of the beer in iron pots or drums, the iron leeching from

the container into the contents as a result of the very low pH generated during the process of fermentation. This iron is in an ionized, highly bioavailable form. The condition is more common in rural areas, where approximately 80% of the African Black population lives and where about two-thirds of adult males consume the traditional beverage. A genetic predisposition allowing an increased absorption of dietary iron in the presence of increased body iron stores is very likely to play a role in the pathogenesis of the condition, although a putative gene has yet to be identified.

Dietary iron overload is complicated by hepatic fibrosis and cirrhosis (in 58% and 10% of patients, respectively). In the early literature the excessive hepatic iron storage was not considered to be a cause of HCC, and no animal model of iron overload-induced HCC was produced. However, between 1996 and 1998 three large studies pathological or clinical concluded that African dietary iron overload was associated with an increased risk of malignant transformation of hepatocytes[77-79].

This observation has recently been supported by the induction of HCC in Wistar albino rats fed an iron-supplemented diet[80]. In the animal model, the iron accumulated in hepatocytes and macrophages, a pattern similar to that seen in African dietary iron overload, and by 16 mo the animals were heavily iron overloaded. At 20 mo iron-free altered hepatic foci were present in many of the animals, and by 28 mo these foci had become more plentiful and had changed in character, becoming indistinguishable from the iron-free preneoplastic nodules described by Deugnier and colleagues in patients with HFE hereditary hemochromatosis who went on to develop HCC[81]. The nodules had clear-cut boundaries, showed an expansive pattern with thickened trabeculae in a deranged pattern causing compression of the surrounding parenchyma, small and large cell dysplasia, and iron-positive intracytoplasmic globules. Further evidence of a proliferative preneoplastic lesion was provided by positive staining for placental glutathione sulphydryl transferase[82]. Moreover, the nodules showed additional features that would be considered dysplastic in the human liver, namely, an increased nuclear/cytoplasmic ratio and Mallory-like inclusions[83]. HCC was seen at 32 mo. Neither fibrosis nor cirrhosis was present in the rat livers, indicating that excessive hepatic iron can be directly hepatocarcinogenic.

The mechanisms by which excess iron induces malignant transformation of hepatocytes have yet to be fully characterized. The most important mechanism appears to be the generation of reactive oxygen species and oxidative stress, which leads to lipid peroxidation of unsaturated fatty acids in membranes of cells and organelles[84]. Cytotoxic by-products of lipid peroxidation, such as malondialdehyde and 4-hydroxy-2'-nonenol, are produced and these impair cellular function and protein synthesis, and damage DNA. Deoxyguanosine residues in DNA are also hydroxylated by reactive oxygen intermediates to form 8-hydroxy-2'-deoxyguanosine, a major promutagenic adduct that causes G:C to T:A transversions and DNA unwinding and strand breaks.

Subsequent studies in the same animal model have shown a synergistic interaction between iron overload and exposure to AFB_1[85] and iron overload and ingestion of alcohol[86] in hepatic mutagenesis.

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