REVIEW

Hepatocellular Carcinoma: From Gene to Public Health

*R. Montesano, P. Hainaut, C. P. Wild**

Liver diseases associated with chronic hepatitis B virus (HBV) infection, including hepatocellular carcinoma, account for more than 1 million deaths annually worldwide. In addition to HBV infection, other risk factors are involved in the etiology of hepatocellular carcinoma and, among these, dietary exposure to the carcinogenic aflatoxins is of particular importance in certain regions of southeast Asia and sub-Saharan Africa. The relative contributions of these two risk factors and the mechanism of the interaction between them in the pathogenesis of hepatocellular carcinoma are still poorly understood. The recently developed individual biochemical and molecular markers of aflatoxin exposure, i.e., aflatoxin–albumin adducts in blood and a specific GC to TA transversion mutation in codon 249 of the p53 gene (249[ser] p53 mutation) in hepatocellular carcinomas, permit a better quantitative estimation of aflatoxin exposure in different populations of the world. A comprehensive summary of the data from our laboratory and the literature, based on a large number (>1000) of individual cases of hepatocellular carcinoma, is presented here and shows the following: 1) A high level and high prevalence of exposure to aflatoxins occur in West Africa, Mozambique, and some regions of China; 2) a high prevalence of the 249[ser] p53 mutation is detected in these countries; and 3) hepatocellular carcinomas from countries with low or no exposure to aflatoxins show a very low prevalence of the 249[ser] p53 mutation and distinctly different p53 mutation spectra, probably indicating different etiologies. Experimental and epidemiologic studies demonstrate an interaction between HBV infection and aflatoxins in hepatocarcinogenesis. The relevance of the biochemical/molecular markers of aflatoxin exposure, HBV vaccination, and the reduction of aflatoxin exposure, in addition to the interaction between HBV infection and other risk factors in liver carcinogenesis, are discussed with regard to the implementation of measures for primary prevention. [J Natl Cancer Inst 1997;89:1844–51]

Vital registration data indicate that adult mortality is higher in developing countries than in industrialized countries and is an important target for the implementation of public health intervention measures *(1)*. It has been estimated that, of a total of 50 million deaths in the world in 1990, 10 million occurred in individuals in the adult age range. Liver diseases associated with chronic hepatitis B virus (HBV) infection, including hepatocel-lular carcinoma, account for more than 1 million deaths annually *(2)*. In addition to HBV infection, other risk factors are involved in the etiology of hepatocellular carcinoma *(3)*, and, among these, dietary exposure to the carcinogenic aflatoxins is of particular importance in certain regions of southeast Asia and sub-Saharan Africa *(4)*.

Aflatoxins are produced by *Aspergillus parasiticus* (aflatoxins B_1, B_2, G_1, and G_2) and *Aspergillus flavus* (aflatoxins B_1 and B_2) and occur, therefore, as mixtures in foods; aflatoxin B_1 is the major component of these mixtures. Aflatoxin M_1, a hydroxyl-ated metabolite of aflatoxin B_1, is also found in milk. Aflatoxin B_1 and, to a lesser degree, aflatoxins G_1 and M_1 are the most biologically active aflatoxins by virtue of their activation to a reactive epoxide form, a reaction that does not occur with afla-toxins B_2 and G_2 *(4)*. Because aflatoxins occur naturally as mixtures in the diet, we generally refer to them collectively in this review unless studies have used a specific compound, in which case the compound is identified.

The relative contributions and the mechanism of interaction between aflatoxins and HBV infection in the etiopathogenesis of hepatocellular carcinoma are still poorly understood. Recently, individual biochemical and molecular markers of aflatoxin exposure, namely, DNA adducts and protein adducts and aflatoxin metabolites in body fluids *(5,6)*, have been developed, and an AGG to AGT mutation affecting the third nucleotide of codon 249 in the p53 gene (249[ser] p53 mutation) in hepatocellular carcinoma *(7,8)* has been specifically associated with exposure to aflatoxins. These findings, together with the previously available serologic markers of HBV infection, have provided valuable tools in molecular epidemiologic studies designed to address this issue. Our review describes the existing data on these markers and discusses their contribution both to understanding the nature of the interaction between HBV infection and afla-toxin exposure in the molecular pathogenesis of hepatocellular

Affiliations of authors: R. Montesano, P. Hainaut, Unit of Mechanisms of Carcinogenesis, International Agency for Research on Cancer, Lyon, France; C. P. Wild, Molecular Epidemiology Unit, Research School of Medicine, University of Leeds, U.K.

Correspondence to: R. Montesano, M.D., Ph.D., Unit of Mechanisms of Carcinogenesis, IARC, 150 cours Albert-Thomas, 69372 Lyon Cedex 08, France. E-mail: ⟨Montesano@iarc.fr⟩

See ''Notes'' following ''References.''

carcinoma and to implementing primary prevention measures to reduce mortality from this cancer.

Aflatoxin Exposure Worldwide

Measurement of aflatoxin exposure on the basis of food analysis is problematic, both because a variety of dietary items can be the sources of aflatoxins and because of difficulties in determining how representative the sampling of the dietary components is at the population level. Furthermore, estimation of human exposure also requires a knowledge of the patterns of consumption of contaminated foods within a given region or population and of seasonal and annual fluctuations in aflatoxin levels. Because of these difficulties, the majority of countries do not have comprehensive data on population exposures to aflatoxins; this is particularly true of those populations with the highest exposures. Nevertheless, epidemiologic studies of specific populations have combined measurements of aflatoxins in foods with food intake estimates to provide calculated population exposures *(4)*. These calculated exposures generally range from 10 to 200 ng/kg body weight per day in sub-Saharan Africa and southeast Asia to less than 3 ng/kg body weight per day in the United States.

An alternative basis for estimating dietary exposures is to use individual biomarkers of exposure. These biomarkers provide a more objective and representative measure of aflatoxin exposure, circumventing as they do the above-named difficulties in developing reliable estimates from dietary assessment *(9,10)*. The biomarker field is particularly advanced for the assessment of aflatoxin exposure, with measurement of covalent binding of aflatoxin to albumin and measurement of urinary aflatoxin metabolites having proved valuable in numerous field studies *(5)* and having been shown to be predictive of hepatocellular carcinoma risk in prospective studies *(11,12)*. The aflatoxin–albumin measurements provide information on individual exposure over the previous 2–3 months, based on the half-life of albumin. Experimental data have also shown that this biomarker reflects the formation of the reactive aflatoxin B_1 metabolite and the level of DNA damage occurring in the livers of rats treated with aflatoxin B_1 *(13)*.

Data from a number of different populations, summarized in Fig. 1, demonstrate that, in some populations in southern China and sub-Saharan Africa, all individuals are exposed to aflatoxins at high levels beginning in the perinatal period and continuing throughout the lifespan of the individual. These biomarker data in individuals have been effective in drawing attention to the sheer magnitude of the exposure to these carcinogens in these parts of the world. Seasonal variations in exposure have also been observed, with higher levels found several months after harvest because of the consumption of stored crops *(14)*. The data on individual exposures are broadly in line with the estimated levels of exposure based on food analyses in different parts of the world.

249[ser] p53 Mutations in Hepatocellular Carcinoma

Mutations in the p53 tumor suppressor gene are present in many human cancers *(15)*, and it has been proposed that some of these mutations are fingerprints of past exposure to a given carcinogen *(16–18)*. Hsu et al. *(8)* and Bressac et al. *(7)* have shown that mutation of the third nucleotide in codon 249 in hepatocellular carcinoma is specifically associated with exposure to aflatoxin, and, since then, a considerable amount of data has accumulated in the literature on the occurrence of such p53 mutations in hepatocellular carcinomas in many regions of the world. Experimental studies in bacteria, in cultured cells, and in animals (*see below*) are also supportive of this association.

A total of 316 p53 mutations in various codons have been detected in over 1000 hepatocellular carcinomas, with the prevalence ranging from 25%–32% in Europe, Japan, and Taiwan to 45% in sub-Saharan Africa and certain regions of China. A high frequency of GC to TA transversions has been detected (*see* Table 1 and Fig. 2). It is extraordinary that more than 50% of the GC to TA transversions occur at one base pair, the third nucleotide of codon 249, resulting in the amino acid substitution of serine for arginine. The prevalence of the aflatoxin-specific p53 mutation, 249[ser], varies greatly in hepatocellular carcinomas from different populations of the world, and Table 1 shows the relationship of this mutation to the incidences of hepatocellular

Fig. 1. Level and prevalence of aflatoxin exposure. Data are expressed as pg aflatoxin B_1 (AFB1)–lysine equivalents/mg serum albumin and represent the mean levels in samples with levels above the detection limit of the enzyme-linked immunosorbent assay used (5 pg/mg). Shandong (China), Europe (France and Poland), and Egypt are represented at the detection limit, but no samples were above this level of adduct (0% prevalence). The number of sera analyzed varies per country, and original data are taken from Wild et al. *(5)* and from reference *(90)*.
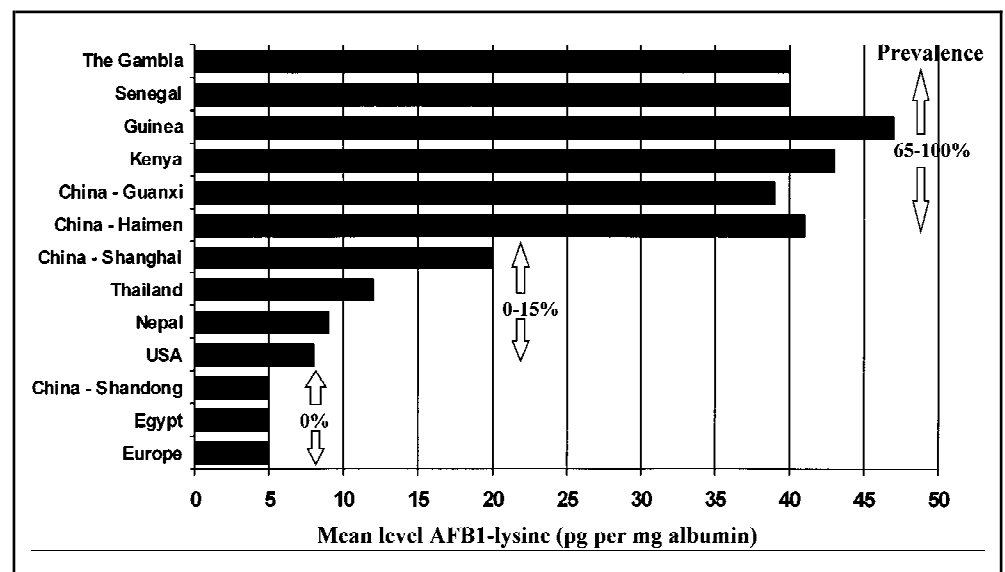
Table 1. Numbers of hepatocellular carcinomas with a 249[ser] p53 mutation and age-standardized rates of hepatocellular carcinoma in countries with low, moderate, and high exposure to aflatoxins*

Location	Age-standardized rates of hepatocellular carcinoma in males/100 000 per year†		
	<20/y	20–40/y	>40/y
High aflatoxin exposure			
Qidong			29 (61)
Mozambique			9 (16)
Senegal			10 (15)
Moderate aflatoxin exposure			
Thailand		1 (15)	
Taiwan		21 (237)	
Mexico		3 (21)	
Shanghai		3 (18)	
Low aflatoxin exposure			
Alaska		0 (13)	
Japan		2 (483)	
Europe, United States, and Australia	1 (168)		

*Data on 249[ser] mutations were obtained from the International Agency for Research on Cancer p53 mutation database *(15)* and from references *(91,92)*; data on age-standardized rates of hepatocellular carcinoma are from reference *(93)*. Aflatoxin exposure information is derived from biomarker data and dietary surveys.

†Numbers in columns refer to the numbers of hepatocellular carcinomas with a 249[ser] p53 mutation, and the numbers in parentheses refer to the total numbers of hepatocellular carcinomas examined.

carcinoma and the levels of exposure to aflatoxin. This particular mutation occurred in 48 cases of a total of 92 hepatocellular carcinomas examined from Qidong (China), Mozambique, and Senegal, whereas 28 of 291 hepatocellular carcinomas with this mutation have been observed in Thailand, Mexico, Shanghai, and Taiwan. In contrast, only one 249[ser] p53 mutation was detected in 168 cases of hepatocellular carcinoma from Europe, the

United States, or Australia. It is informative to correlate aflatoxin exposure, as determined by biomarker measurements (Fig. 1), with the prevalence of this specific mutation in the p53 gene. Populations in southern China (Guanxi, Qidong, and Haimen), West Africa (The Gambia, Senegal, and Guinea–Conakry), and East Africa (Kenya) have the highest recorded levels of exposure, as determined by aflatoxin–albumin adduct measurements, and, as noted above, these countries are among those with the highest prevalence of 249[ser] p53 mutations. The only country with a high prevalence of 249[ser] p53 mutations and for which individual biomarker data are unavailable is Mozambique. However, it is known that food levels of aflatoxins are particularly high in this country (38–184 ng aflatoxin B_1/kg body weight per day) *(4)*. In what can be classified as regions of intermediate exposure on the basis of biomarker data (Thailand, Taiwan, and Shanghai), mutation data reveal an intermediate prevalence of 249[ser] p53 mutations. Dietary analysis for aflatoxins in Mexico suggests a relatively high level of exposure because of the frequent consumption of corn (10–35 g corn/day), and this assessment is supported by a limited analysis of sera from 16 patients with hepatocellular carcinoma. All of the sera were positive for aflatoxin–albumin adducts at high levels *(19)*. In Taiwan, qualitative data on aflatoxin–albumin adducts, obtained by means of an enzyme-linked immunosorbent assay of similar sensitivity to the one that was used to generate the data shown in Fig. 1, revealed a prevalence of 34% of sera positive for this marker *(20)*. Exposure levels to aflatoxin B_1 are low in Europe, the United States, and Egypt and correlate with the low prevalence of 249[ser] p53 mutations in hepatocellular carcinomas from these countries.

When these data are all considered, a clear relationship is observed between the prevalence of 249[ser] p53 mutations, exposure to aflatoxins, and the incidence of hepatocellular carcinoma (Table 1). It should be noted that this analysis is essentially an ecological correlation, and there is a possibility of

Fig. 2. p53 mutation spectra in hepatocellular carcinomas from different regions of the world. Data were obtained from the International Agency for Research on Cancer p53 mutation database *(15)*. The numbers in parentheses refer to the numbers of hepatocellular carcinomas examined, and the numbers in the small boxes refer to the total number of p53 mutations detected (exons 5 through 8) in hepatocellular carcinomas from each region or country. The large box contains the key for the mutation types, which are denoted in the form of numbers, 1 through 8, on the *x* axis for each region or country. The key is read as follows: 1, AT to GC transversion; 2, AT to GC transition; 3, AT to TA transversion; 4, GC to AT transition; 5, GC to AT transition at CpG islands; 6, GC to CG transversion; 7, GC to TA transversion; and 8, insertion or deletion. "Others" refer collectively to Mexico, Shanghai, Beijing, Thailand, and Transkei.
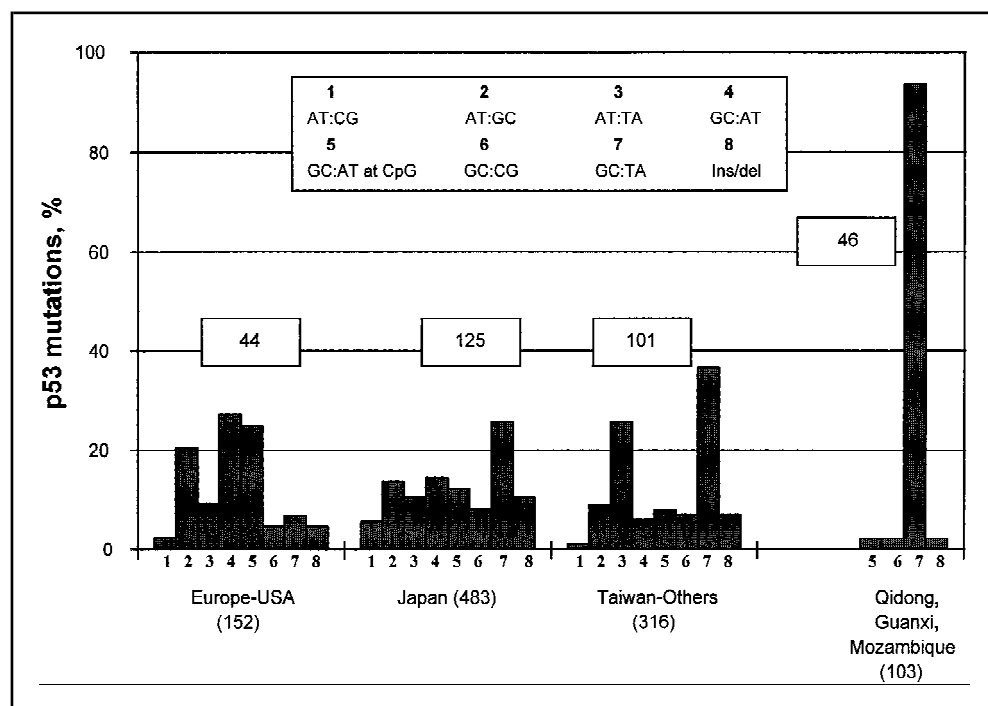
confounding caused by concomitant exposures to other environmental factors. A correlation at the individual level between aflatoxin exposure and the presence of the 249[ser] p53 mutation in hepatocellular carcinoma would provide a better opportunity to control for such confounding and would provide more direct evidence of a causal association. Attempts have been made to establish such a correlation by measuring aflatoxin B_1–DNA adducts in the liver tissue of patients with hepatocellular carcinoma (21,22). However, the limitations of such an approach include the possible influence of the disease state on adduct levels (by alteration of diet or metabolism) and the fact that recent exposure, as measured by the biomarker, may not reflect past exposure. A prospective study design would be more appropriate to examine this question.

The great majority of the hepatocellular carcinoma case patients in the above-mentioned studies were positive for hepatitis B virus surface antigen (HBsAg), a marker of chronic HBV infection. While relatively few patients with hepatocellular carcinoma were HBsAg negative, a higher frequency of 249[ser] p53 mutations did seem to occur in HBsAg-positive patients (23). However, as Eaton and Gallagher (23) have noted, this analysis should be interpreted with caution because of the difficulty in clearly defining the HBV status of patients with hepatocellular carcinoma on the basis of published data. Nevertheless the tendency to higher levels of 249[ser] p53 mutations in HBsAg-positive individuals is suggestive of a synergistic interaction between HBV infection and aflatoxin exposure in the induction of hepatocellular carcinoma. This synergy is in turn consistent with epidemiologic data (11,12) and experimental studies in the woodchuck (24) and in HBV-transgenic mice (25) that show an increased incidence or an earlier onset of tumors in cases of combined exposure. However, it is also possible that the association between the 249[ser] p53 mutation and HBsAg positivity is a fortuitous one, in that areas of high aflatoxin exposure are those with a high prevalence of HBV infection.

In Alaska and Japan, areas with a low exposure to aflatoxins and a low prevalence of 249[ser] p53 mutations (in two of a total of 496 HCC cases of hepatocellular carcinoma; see Table 1), the incidence of hepatocellular carcinoma is, nevertheless, relatively high. This high incidence rate is likely to be associated with other risk factors, namely, a high prevalence of hepatitis C virus (HCV) infection and/or interaction between HBV/HCV infection and alcohol consumption (26,27).

p53 Mutation Spectra in Hepatocellular Carcinoma

As already mentioned, in regions of high exposure to aflatoxins, such as Qidong-Guanxi (China) and Mozambique, a high proportion of the p53 mutations detected in heptocellular carcinomas consisted of GC to TA transversions (43 of 46), and 40 of these were specifically 249[ser] mutations. It is, however, of interest to note (Fig. 2) that, in addition to these 249[ser] mutations, significant differences in the overall p53 mutation spectra are also observed among hepatocellular carcinomas from various regions of the world. In hepatocellular carcinomas from Europe and the United States, a low prevalence (<10%) of GC to TA tranversions and a 20%–30% prevalence of GC to AT transitions are observed together with an increased level of mutations in-

volving AT base pairs. In Japan, a higher prevalence of AT base pair mutations and an increase in overall GC to TA tranversions are seen. It is also interesting that, in hepatocellular carcinomas from Taiwan, a particularly high prevalence of AT to TA tranversions is found. This p53 mutation profile indicates that risk factors other than aflatoxin exposure, such as DNA damage attributable to oxidative free radicals, lipid peroxidation, and alcohol intake (28,29), are involved in generating the mutations detected in different regions of the world.

Experimental Data

The presence of the 249[ser] p53 mutation has been examined in hepatocellular carcinomas induced by aflatoxin B_1 in various experimental systems. Nonhuman primates have a DNA sequence at codons 247–250 that is identical to that found in humans, but the data with respect to 249[ser] p53 mutations following aflatoxin B_1 exposure are uninformative because of the small number of cases examined. In rhesus and cynomolgus monkeys, no 249[ser] p53 mutations were detected in a total of four cases of hepatocellular carcinoma, two of which originated in a single animal; other tumors reported as induced by aflatoxin B_1 in this study (namely, two cholangiocarcinomas, a spindle cell carcinoma of the bile duct, a hemangioendothelial sarcoma of the liver, and an osteogenic sarcoma of the tibia) were also analyzed with negative results (30). The prevalence of 249[ser] p53 mutations in human hepatocellular carcinomas reaches a maximum of 50%–60% (see Table 1); thus, the number of nonhuman primate hepatocellular carcinomas analyzed is too small to ensure the reliability of these negative findings. The ground squirrel, the woodchuck, the duck, and the rat have a different p53 DNA sequence at codons 247–250 than humans, and no 249[ser] p53 mutations were detected in hepatocellular carcinomas (or liver nodules, in the case of the rat) from these animals following exposure to aflatoxin B_1 (31–35). In the rat and the woodchuck, a G:C to T:A transversion at the equivalent of the third nucleotide in codon 249 would, in any case, result in a silent mutation. In summary, because of the small number of hepatocellular carcinomas examined in monkeys and because of the limitations of the other experimental systems used, the experimental data on 249[ser] p53 mutations in hepatocellular carcinomas induced by aflatoxin B_1 do not contradict the observations in human hepatocellular carcinomas.

An additional issue that requires consideration in cross-species comparisons is the role of p53 in carcinogenesis and, particularly, the functional importance of the 249[ser] p53 mutation in hepatocytes. In rats treated with carcinogens, including aflatoxin B_1, there has generally been an absence of p53 mutations in liver tumors, or contradictory findings have been reported. In murine hepatocellular carcinomas, no p53 mutations have been detected (36,37). A high prevalence of p53 mutations was, however, observed in hepatocellular carcinomas induced in rats treated with tamoxifen and 2-acetylaminofluorene (38,39). The 249[ser] p53 mutation may also have a specific functional effect on, for example, mitotic activity in human hepatocytes that is not found in hepatocytes from other species (40). Although these findings indicate that the molecular pathogenesis of liver cancer in rodents and humans may proceed through different pathways, further genetic analysis with appropriate tech-

niques is required to substantiate the conclusion of such a difference.

The nature of the mutations induced by aflatoxin B_1 has been examined in several systems, including human cells. These data show that the predominant mutations are G:C to T:A transversions *(41–45)*, that the aflatoxin B_1–N7–G adduct is the primary DNA adduct responsible for this mutation *(46)*, and that the nucleotide sequence influences the target for aflatoxin B_1 binding *(44,47,48)*. There is also evidence that GC to TA transversions are targeted to codon 249 of p53, in comparison with surrounding codons, both in plasmid DNA treated with aflatoxin B_1 *in vitro (49)* and in human hepatocytes treated with aflatoxin B_1 in culture *(50)*. In addition, the complex balance of cytochrome P450-, epoxide hydrolase-, and glutathione *S*-transferase-mediated metabolism of aflatoxin B_1 and DNA repair processes is an important determinant in the formation of DNA damage and mutations caused by this carcinogen *(51)*. The expression of the enzymes in these metabolic and repair pathways is critical in determining cross-species differences in the formation of aflatoxin–DNA and aflatoxin–protein adducts as well as in the susceptibility to aflatoxin-induced carcinogenesis *(52)*. The frequency of different polymorphisms in some of the aflatoxin-metabolizing enzymes also varies within the human population (e.g., between ethnic groups), and the impact of these variations on the susceptibility to aflatoxins at the individual and population level is still poorly documented *(53)*.

Mutations in codon 249 of p53 are not exclusive to hepatocellular carcinoma, and such mutations account for about 5% of all p53 mutations in human cancers, as reported in the International Agency for Research on Cancer p53 mutation database *(15)*. It is of interest to note that the two carcinogens, benzo[*a*]-pyrene and aflatoxin B_1, produce within this codon different substitutions for arginine. Benzo[*a*]pyrene produces a substitution of methionine for arginine (AGG to ATG) *(54)*, whereas aflatoxin B_1 is associated with an arginine-to-serine substitution. This difference probably reflects differential binding of these carcinogens to a given DNA sequence and differential repair of these DNA adducts.

Arginine 249 plays an essential structural role in the folding of the DNA-binding domain of p53 *(55)*. In contrast with Arg 248 and Arg 273, the most commonly mutated arginines in human cancers, Arg 249 does not make direct contact with specific DNA bases, but it belongs to the highly critical L3 loop and is adjacent to Arg 248, which contacts DNA in the minor groove. Arg 249 makes contacts with four other amino acid residues distributed in different secondary structural motifs, i.e., His 162 and Glu 171 (both in L2 loop) and Met 246 and Gly 245 (both in L3 loop) *(55)*. Analysis of the tertiary structure of p53 indicates that these contacts contribute to the stabilization of the L3 loop and to its correct orientation with respect to the DNA-binding surface. Mutation of this residue is expected to have major structural consequences and to destabilize the domain of p53 that makes contacts with the minor groove of DNA [Hainaut P: unpublished results; *(56)*]. Experimentally, the mutant Ser 249 polypeptide has been shown to have a significant dominant-negative effect on wild-type p53 in several cell types, including one hepatocellular carcinoma cell line *(40)*. These structural and functional implications of mutations at codon 249 may contribute to the explanation of why these mutations are specifically selected in hepatocellular carcinoma and why they are retained during cancer development.

Mechanistic Considerations

The data summarized here clearly indicate the strong association between aflatoxin exposure and the occurrence of the 249^{ser} p53 mutation in hepatocellular carcinoma originating in certain areas of China and sub-Saharan Africa. This association is supported by the use of biomarker data on aflatoxin exposure, which provide a more reliable and a more precise picture than the dietary data used previously. It is, however, apparent that genetic alterations in genes other than the p53 tumor suppressor gene, as well as altered expression of various cellular proteins, also occur in the natural history of hepatocellular carcinoma *(36,57–60)*.

The types of oncogenes or tumor suppressor genes involved and the temporal occurrence of alterations (early versus late) in these genes vary greatly among different types of tumors *(61)*. Inactivation of the p53 gene seems to occur late in the development of colon cancers *(62)* and early in glioblastomas *(63)* and esophageal cancers *(64)*. It is reasonable to assume that the temporal occurrence of these genetic, as well as epigenetic, events may differ in hepatocellular carcinomas with different etiologies. In the case of hepatocellular carcinomas in regions of high exposure to aflatoxins, there is sound evidence that the 249^{ser} p53 mutation is an early event, as shown by the presence of this mutation in normal hepatic tissue from patients with hepatocellular carcinoma in regions with high exposure to aflatoxin B_1 *(65)*. This observation is consistent with data from The Gambia *(66)* showing that exposure to aflatoxin B_1 occurs even during the perinatal period and continues for the entire lifespan in the great majority of the population. The exposure to this carcinogen and the concomitant high proportion of HBV chronic carriers are probably associated with the early occurrence (already at the age of 12–15 years) and the peak incidence in young adults (aged 35–45 years) of hepatocellular carcinoma reported in The Gambia, Guinea–Conakry, Zimbabwe, and Qidong and Guanxi (China) *(11,67–70)*. Experimental studies in HBV transgenic mice have shown that transplacental aflatoxin B_1 exposure is associated with the occurrence of alterations in minisatellite DNA sequences detected in hepatocellular carcinomas developing in adult mice *(71)*. In addition, the possibility that HBV infection alters aflatoxin metabolism has been suggested from studies in HBV transgenic mice *(72,73)*. The mechanism by which HBV infection is responsible for the induction of hepatocellular carcinoma is still unclear. The development of hepatocellular carcinoma as a consequence of chronic HBV infection has been associated in only rare cases with insertional mutagenesis *(74)*. Some evidence indicates that the HBV X protein may bind to and inactivate the p53 protein *(75–79)*. Another mechanism, and at present the most plausible, is that recurrent liver cell proliferation occurring among HBV chronic carriers is the critical rate-limiting factor favoring the selective clonal development of cells mutated by aflatoxin B_1 in certain geographic regions or mutated by other exogenous or endogenous sources of cellular DNA damage in other regions *(18,80)*. Further evidence supporting the role of cell proliferation is provided by the fact that HCV infection results in chronic hepatitis and cirrhosis with an

increased rate of cell death and cell division and the fact that HCV infection is associated, as is the case with HBV, with a high risk of hepatocellular carcinoma *(26)*. While HBV is a DNA virus, HCV is an RNA virus, and there is no evidence of integration of HCV sequences into cellular DNA or for an HCV-encoded transactivating protein.

Cancer Prevention

The availability of biologic markers of exposure to aflatoxin and to HBV infection and an understanding of their role in the pathogenesis of hepatocellular carcinoma have a direct bearing on the assessment of the relative importance of the different causative agents and, consequently, on the implementation of measures for primary prevention. A program of vaccination against HBV in The Gambia was progressively introduced from 1986 as part of the World Health Organization's Expanded Programme of Immunisation, and the results to date show a high degree of protection against primary HBV infection and the chronic carrier state *(68,81,82)*. The next decade will provide evidence of the degree of protection against the development of hepatocellular carcinoma. The study in The Gambia has also shown the high cost-effectiveness of HBV vaccination in preventing deaths from chronic liver diseases or liver cancer *(83)*. Thus, economic considerations should not be an impediment to integrating HBV vaccination into national programs of immunization in regions of high prevalence of HBV carriers, as recommended by the World Health Organization *(2)*.

It remains to be clarified what proportion of hepatocellular carcinomas is attributable solely to aflatoxin exposure and how much can be attributed to an interaction with HBV infection. If the latter is the major contributor, HBV vaccination may be sufficient to reduce the incidence of hepatocellular carcinoma associated with aflatoxin exposure. At the same time, there are some 300–400 million HBV chronic carriers currently present in the world who will continue to be at risk of hepatocellular carcinoma, and high proportions of these are exposed to aflatoxins. In addition, aflatoxins may be relevant to diseases other than cancer *(9,84)*. Thus, in regions such as West Africa and in some regions of China with high exposures to aflatoxins, implementing measures to reduce exposure to this class of potent carcinogens must be a priority.

Intervention measures against aflatoxins can be targeted at the individual or the community level. At the individual level, in societies where the dietary staple is contaminated with aflatoxins, avoidance of the exposure by dietary change is unrealistic. An alternative strategy would be chemoprevention, using compounds that limit the deleterious consequences of aflatoxins once ingested. This approach has been evaluated in experimental models with the anti-schistosomal drug oltipraz, which modulates aflatoxin detoxification *(85)*, and a clinical trial has been conducted in the People's Republic of China using this compound *(86)*. The realistic possibility of applying this chemopreventive approach at the population level is still doubtful. More promising as a long-term strategy for intervention at the community level in developing countries are attempts to limit the formation of aflatoxins before or after harvest. These attempts include sorting methods, biocontrol methods, and chemical and physical methods for the destruction and degradation of aflatox-

ins *(87)*. The various attempts in this direction have not been successful for foods for human consumption because of the chemical stability of the aflatoxin molecule under normal cooking conditions and the consequence that decontamination or detoxification procedures render the food unsuitable for human consumption. Nevertheless, there are data that indicate that simple, low technology pre- and post-harvest improvements in agricultural and food storage practices might result in lower levels of aflatoxin contamination in human foods *(88)*. A further promising alternative in the long term is the development of transgenic plants that are resistant to infestation by *Aspergillus* or are resistant to aflatoxin biosynthesis *(89)*. Validated markers of human exposure to aflatoxins, such as aflatoxin–albumin adducts and the 249^{ser} p53 mutation, would be valuable surrogate end points in trials conducted to test the efficiency of the above intervention procedures.

References

(1) Murray CJ, Feachem RG. Adult mortality in developing countries. Trans R Soc Trop Med Hyg 1990;84:1–2.

(2) Kane M. Global programme for control of hepatitis B infection. Vaccine 1995;13 Suppl 1:S47–9.

(3) Tomatis L. Cancer: causes, occurrence and control. In: IARC Scientific Publications No. 100. Lyon (France): IARC, 1990.

(4) Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monogr Eval Carcinog Risks Hum, vol 56. Lyon (France): IARC, 1996.

(5) Wild CP, Jansen LA, Cova L, Montesano R. Molecular dosimetry of aflatoxin exposure: contribution to understanding the multifactorial etiopathogenesis of primary hepatocellular carcinoma with particular reference to hepatitis B virus. Environ Health Perspect 1993;99:115–22.

(6) Groopman JD. Molecular dosimetry methods for assessing human aflatoxin exposures. In: Eaton DA, Groopman JD, editors. The toxicology of aflatoxins: human health, veterinary and agricultural significance. New York: Academic Press, 1994:259–79.

(7) Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa [see comments]. Nature 1991;350:429–31.

(8) 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.

(9) Hall AJ, Wild CP. Epidemiology of aflatoxin related disease. In: Eaton DA, Groopman JD, editors. The toxicology of aflatoxins: human health, veterinary and agricultural significance. New York: Academic Press, 1994: 233–58.

(10) Wild CP, Pisani P. Carcinogen-DNA and -protein adducts in molecular epidemiology. In: Toniolo P, Boffetta P, Shuker D, Rothman N, Hulka B, Pearce N, editors. Application of biomarkers to cancer epidemiology. IARC Sci Publ 1997;142:143–58.

(11) Qian GS, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, et al. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers Prev 1994;3:3–10.

(12) Wang JS, Qian GS, Zarba A, He X, Zhu YR, Zhang BC, et al. Temporal patterns of aflatoxin–albumin adducts in hepatitis B surface antigen-positive and antigen-negative residents of Daxin, Qidong County, People's Republic of China. Cancer Epidemiol Biomarkers Prev 1996;5:253–61.

(13) Wild CP, Garner RC, Montesano R, Tursi F. Aflatoxin B1 binding to plasma albumin and liver DNA upon chronic administration to rats. Carcinogenesis 1986;7:853–8.

(14) Wild CP, Jiang YZ, Allen SJ, Jansen LA, Hall AJ, Montesano R. Aflatoxin–albumin adducts in human sera from different regions of the world. Carcinogenesis 1990;11:2271–4.

(15) Hainaut P, Soussi T, Shomer B, Hollstein M, Greenblatt M, Hovig E, et al. Database of p53 gene somatic mutations in humans tumors and cell lines: updated compilation and future prospects. Nucleic Acids Res 1997;25: 151–7.

(16) Esteve A, Sorlie T, Martel-Planche G, Hollstein M, Kusters I, Lewalter J, et al. Screening for p53 gene mutations in archived tumors of workers occupationally exposed to carcinogens: examples from analysis of bladder tumors. J Occup Environ Med 1995;37:59–68.

(17) Harris CC. Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res 1991;51(18 Suppl):5023s–5044s.

(18) Harris CC. The 1995 Walter Hubert Lecture—molecular epidemiology of human cancer: insights from the mutational analysis of the p53 tumour-suppressor gene. Br J Cancer 1996;73:261–9.

(19) Soini Y, Chia SC, Bennett WP, Groopman JD, Wang JS, DeBenedetti VM, et al. An aflatoxin-associated mutational hotspot at codon 249 in the p53 tumor suppressor gene occurs in hepatocellular carcinomas from Mexico. Carcinogenesis 1996;17:1007–12.

(20) Wang LY, Hatch M, Chen CJ, Levin B, You SL, Lu SN, et al. Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. Int J Cancer 1996;67:620–5.

(21) Hollstein MC, Wild CP, Bleicher F, Chutimataewin S, Harris CC, Srivatanakul P, et al. p53 mutations and aflatoxin B_1 exposure in hepatocellular carcinoma patients from Thailand. Int J Cancer 1993;53:51–5.

(22) Hsieh DP, Atkinson DN. Recent aflatoxin exposure and mutation at codon 249 of the human p53 gene: lack of association. Food Addit Contam 1995; 12:421–4.

(23) Eaton DL, Gallagher EP. Mechanisms of aflatoxin carcinogenesis. Annu Rev Pharmacol Toxicol 1994;34:135–72.

(24) Bannasch P, Khoshkhou NI, Hacker HJ, Radaeva S, Mrozek M, Zillmann U, et al. Synergistic hepatocarcinogenic effect of hepadnaviral infection and dietary aflatoxin B_1 in woodchucks. Cancer Res 1995;55:3318–30.

(25) Sell S, Hunt JM, Dunsford HA, Chisari FV. Synergy between hepatitis B virus expression and chemical hepatocarcinogens in transgenic mice. Cancer Res 1991;51:1278–85.

(26) Hepatitis viruses. IARC Monogr Eval Carcinog Risks Hum, vol 59. Lyon (France): IARC, 1994.

(27) Alcohol drinking. IARC Monogr Eval Carcinog Risks Hum, vol 44. Lyon (France): IARC, 1988.

(28) Hagen TM, Huang S, Curnutte J, Fowler P, Martinez V, Wehr CM, et al. Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. Proc Natl Acad Sci U S A 1994;91:12808–12.

(29) Bartsch H, Barbin A, Marion MJ, Nair J, Guichard Y. Formation, detection, and role in carcinogenesis of ethenobases in DNA. Drug Metab Rev 1994; 26:349–71.

(30) Fujimoto Y, Hampton LL, Luo LD, Wirth PJ, Thorgeirsson SS. Low frequency of p53 gene mutation in tumors induced by aflatoxin B_1 in nonhuman primates. Cancer Res 1992;52:1044–6.

(31) Hulla JE, Chen ZY, Eaton DL. Aflatoxin B_1-induced rat hepatic hyperplastic nodules do not exhibit a site-specific mutation within the p53 gene. Cancer Res 1993;53:9–11.

(32) Duflot A, Hollstein M, Mehrotra R, Trepo C, Montesano R, Cova L. Absence of p53 mutation at codon 249 in duck hepatocellular carcinomas from the high incidence area of Qidong (China). Carcinogenesis 1994;15:1353–7.

(33) Rivkina M, Cote PJ, Robinson WS, Tennant BC, Marion PL. Absence of mutations in the p53 tumor suppressor gene in woodchuck hepatocellular carcinomas associated with hepadnavirus infection and intake of aflatoxin B_1. Carcinogenesis 1996;17:2689–94.

(34) Rivkina MB, Cullen JM, Robinson WS, Marion PL. State of the p53 gene in hepatocellular carcinomas of ground squirrels and woodchucks with past and ongoing infection with hepadnaviruses. Cancer Res 1994;54:5430–7.

(35) Tokusashi Y, Fukuda I, Ogawa K. Absence of p53 mutations and various frequencies of Ki-ras exon 1 mutations in rat hepatic tumors induced by different carcinogens. Mol Carcinog 1994;10:45–51.

(36) Grisham JW. Interspecies comparison of liver carcinogenesis: implications for cancer risk assessment. Carcinogenesis 1997;18:59–81.

(37) Montesano R, Kirby G. Chemical carcinogens in human liver cancer. In: Brechot C, editor. Primary liver cancer: etiological and progression factors. Boca Raton (FL): CRC Press, 1994:55–77.

(38) Ho YS, Cheng HT, Wang YJ, Lin JK. p53 gene mutational spectra in hepatocellular carcinomas induced by 2-acetylaminofluorene and N-nitroso-2-acetylaminofluorene in rats. Mol Carcinog 1995;13:182–90.

(39) Vancutsem PM, Lazarus P, Williams GM. Frequent and specific mutations of the rat p53 gene in hepatocarcinomas induced by tamoxifen. Cancer Res 1994;54:3864–7.

(40) Ponchel F, Puisieux A, Tabone E, Michot JP, Froschl G, Morel AP, et al. Hepatocarcinoma-specific mutant p53–249^{ser} induces mitotic activity but has no effect on transforming growth factor beta 1-mediated apoptosis. Cancer Res 1994;54:2064–8.

(41) Trottier Y, Waithe WI, Anderson A. Kinds of mutations induced by aflatoxin B_1 in a shuttle vector replicating in human cells transiently expressing cytochrome P4501A2 cDNA. Mol Carcinog 1992;6:140–7.

(42) Cariello NF, Cui L, Skopek TR. In vitro mutational spectrum of aflatoxin B_1 in the human hypoxanthine guanine phosphoribosyltransferase gene. Cancer Res 1994;54:4436–41.

(43) Levy DD, Groopman JD, Lim SE, Seidman MM, Kraemer KH. Sequence specificity of aflatoxin B_1-induced mutations in a plasmid replicated in xeroderma pigmentosum and DNA repair proficient human cells. Cancer Res 1992;52:5668–73.

(44) Dycaico MJ, Stuart GR, Tobal GM, de Boer JG, Glickman BW, Provost GS. Species-specific differences in hepatic mutant frequency and mutational spectrum among lambda/lacI transgenic rats and mice following exposure to aflatoxin B_1. Carcinogenesis 1996;17:2347–56.

(45) Foster PL, Eisenstadt E, Miller JH. Base substitution mutations induced by metabolically activated aflatoxin B_1. Proc Natl Acad Sci U S A 1983;80:2695–8.

(46) Bailey EA, Iyer RS, Stone MP, Harris TM, Essigmann JM. Mutational properties of the primary aflatoxin B_1–DNA adduct. Proc Natl Acad Sci U S A 1996;93:1535–9.

(47) Benasutti M, Ejadi S, Whitlow MD, Loechler EL. Mapping the binding site of aflatoxin B_1 in DNA: systematic analysis of the reactivity of aflatoxin B_1 with guanines in different DNA sequences. Biochemistry 1988;27:472–81.

(48) Muench KF, Misra RP, Humayun MZ. Sequence specificity in aflatoxin B_1–DNA interactions. Proc Natl Acad Sci U S A 1983;80:6–10.

(49) Puisieux A, Lim S, Groopman J, Ozturk M. Selective targeting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. Cancer Res 1991;51:6185–9.

(50) Aguilar F, Hussain SP, Cerutti P. Aflatoxin B_1 induces the transversion of G→T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. Proc Natl Acad Sci U S A 1993;90:8586–90.

(51) Guengerich FP, Johnson WW, Ueng YF, Yamazaki H, Shimada T. Involvement of cytochrome P450, glutathione S-transferase, and epoxide hydrolase in the metabolism of aflatoxin B_1 and relevance to risk of human cancer. Environ Health Perspect 1996;104 Suppl 3:557–62.

(52) Wild CP, Hasegawa R, Barraud L, Chutimataewin S, Chapot B, Ito N, et al. Aflatoxin–albumin adducts: a basis for comparative carcinogenesis between animals and humans. Cancer Epidemiol Biomarkers Prev 1996;5:179–89.

(53) McGlynn KA, Rosvold EA, Lustbader ED, Hu Y, Clapper ML, Zhou T, et al. Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B_1. Proc Natl Acad Sci U S A 1995;92:2384–7.

(54) Denissenko MF, Pao A, Tang M, Pfeifer GP. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. Science 1996;274:430–2.

(55) Cho Y, Gorina S, Jeffrey PD, Pavletich NP. Crystal structure of a p53 tumor suppressor–DNA complex: understanding tumorigenic mutations. Science 1994;265:346–55.

(56) Adler V, Pincus MR, Minamoto T, Fuchs SY, Bluth MJ, Brandt-Rauf PW, et al. Conformation-dependent phosphorylation of p53. Proc Natl Acad Sci U S A 1997;94:1686–91.

(57) Katagiri T, Nakamura Y, Miki Y. Mutations in the BRCA2 gene in hepatocellular carcinomas. Cancer Res 1996;56:4575–7.

(58) Murakami Y, Hayashi K, Hirohashi S, Sekiya T. Aberrations of the tumor suppressor p53 and retinoblastoma genes in human hepatocellular carcinomas. Cancer Res 1991;51:5520–5.

(59) Tabor E. Tumor suppressor genes, growth factor genes, and oncogenes in hepatitis B virus-associated hepatocellular carcinoma. J Med Virol 1994; 42:357–65.

(60) Tahara H, Nakanishi T, Kitamoto M, Nakashio R, Shay JW, Tahara E, et al. Telomerase activity in human liver tissues: comparison between chronic liver disease and hepatocellular carcinomas. Cancer Res 1995;55:2734–6.

(61) Murray A, Hunt T, editors. The cell cycle: an introduction. Oxford: Oxford University Press, 1993.

(62) Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.

(63) Lasko D, Cavenee W, Nordenskjold M. Loss of constitutional heterozygosity in human cancer. Annu Rev Genet 1991;25:281–314.

(64) Montesano R, Hollstein M, Hainaut P. Genetic alterations in esophageal cancer and their relevance to etiology and pathogenesis: a review. Int J Cancer 1996;69:225–35.

(65) Aguilar F, Harris CC, Sun T, Hollstein M, Cerutti P. Geographic variation of p53 mutational profile in nonmalignant human liver. Science 1994;264:1317–9.

(66) Wild CP, Rasheed FN, Jawla MF, Hall AJ, Jansen LA, Montesano R. *In-utero* exposure to aflatoxin in west Africa [letter]. Lancet 1991;337:1602.

(67) Bah E, Hall AJ, Inskip HM. The first 2 years of the Gambian National Cancer Registry. Br J Cancer 1990;62:647–50.

(68) Viviani S, Jack A, Bah E, Montesano R. Hepatocellular carcinoma: a preventable cancer. Epidemiol Prev 1997;21:129–36.

(69) Koulibaly M, Kabba IS, Cisse A, Diallo SB, Diallo MB, Keita N, et al. Cancer incidence in Conakry, Guinea: first results from the Cancer Registry 1992–1995. Int J Cancer 1997;70:39–45.

(70) Parkin DM, Vizcaino AP, Skinner ME, Ndhlovu A. Cancer patterns and risk factors in the African population of southwestern Zimbabwe, 1963–1977. Cancer Epidemiol Biomarkers Prev 1994;3:537–47.

(71) Kaplanski C, Chisari FV, Wild CP. Minisatellite rearrangements are increased in liver tumours induced by transplacental aflatoxin B_1 treatment of hepatitis B virus transgenic mice, but not in spontaneously arising tumours. Carcinogenesis 1997;18:633–9.

(72) Kirby GM, Chemin I, Montesano R, Chisari FV, Lang MA, Wild CP. Induction of specific cytochrome P450s involved in aflatoxin B_1 metabolism in hepatitis B virus transgenic mice. Mol Carcinog 1994;11:74–80.

(73) Chemin I, Takahashi S, Belloc C, Lang MA, Ando K, Guidotti LG, et al. Differential induction of carcinogen metabolizing enzymes in a transgenic mouse model of fulminant hepatitis. Hepatology 1996;24:649–56.

(74) Graef E, Caselmann WH, Wells J, Koshy R. Insertional activation of mevalonate kinase by hepatitis B virus DNA in a human hepatoma cell line. Oncogene 1994;9:81–7.

(75) Puisieux A, Ji J, Guillot C, Legros Y, Soussi T, Isselbacher K, et al. p53-mediated cellular response to DNA damage in cells with replicative hepatitis B virus. Proc Natl Acad Sci U S A 1995;92:1342–6.

(76) Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc Natl Acad Sci U S A 1994;91:2230–4.

(77) Henkler F, Waseem N, Golding MH, Alison MR, Koshy R. Mutant p53 but not hepatitis B virus X protein is present in hepatitis B virus-related human hepatocellular carcinoma. Cancer Res 1995;55:6084–91.

(78) Truant R, Antunovic J, Greenblatt J, Prives C, Cromlish JA. Direct interaction of the hepatitis B virus HBx protein with p53 leads to inhibition by HBx of p53 response element-directed transactivation. J Virol 1995;69:1851–9.

(79) Greenblatt MS, Feitelson MA, Zhu M, Bennett WP, Welsh JA, Jones R, et al. Integrity of p53 in hepatitis Bxantigen-positive and -negative hepatocellular carcinomas. Cancer Res 1997;57:426–32.

(80) Harris CC, Sun T. Multifactoral etiology of human liver cancer. Carcinogenesis 1984;5:697–701.

(81) Hall AJ, Inskip HM, Loik F, Chotard J, Jawara M, Vall Mayans M, et al. Hepatitis B vaccine in the expanded programme of immunisation: The Gambian experience. The Gambia Hepatitis Study Group. Lancet 1989;1:1057–9.

(82) Fortuin M, Chotard J, Jack AD, Maine NP, Mendy M, Hall AJ, et al. Efficacy of hepatitis B vaccine in the Gambian expanded programme on immunisation. Lancet 1993;341:1129–31.

(83) Hall AJ, Roberston RL, Crivelli PE, Lowe Y, Inskip H, Snow SK, et al. Cost-effectiveness of hepatitis B vaccine in The Gambia. Trans R Soc Trop Med Hyg 1993;87:333–6.

(84) Denning DW. Aflatoxin and human disease. Adverse Drug React Acute Poisoning Rev 1987;6:175–209.

(85) Roebuck BD, Liu YL, Rogers AE, Groopman JD, Kensler TW. Protection against aflatoxin B_1-induced hepatocarcinogenesis in F344 rats by 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione (oltipraz): predictive role for short-term molecular dosimetry. Cancer Res 1991;51:5501–6.

(86) Jacobson LP, Zhang BC, Zhu YR, Wang JB, Wu Y, Zhang QN, et al. Oltipraz chemoprevention trial in Qidong, People's Republic of China: study design and clinical outcomes. Cancer Epidemiol Biomarkers Prev 1997;6:257–65.

(87) Phillips TD, Clement BA, Park DL. Approaches to reduction of aflatoxins in foods and feeds. In: Eaton DA, Groopman JD, editors. The toxicology of aflatoxins: human health, veterinary and agricultural significance. New York: Academic Press, 1994:383–406.

(88) Compton JA, Tyler PS, Hindmarsh PS, Golob P, Boxall RA, Haines CP. Reducing losses in small farm grain storage in the tropics. Trop Sci 1993;33:283–318.

(89) Bhatnagar D, Cleveland TE, Cotty PJ. Mycological aspects of aflatoxin formation. In: Eaton DA, Groopman JD, editors. The toxicology of aflatoxins: human health, veterinary and agricultural significance. New York: Academic Press, 1994:327–46.

(90) Yu SZ. Primary prevention of hepatocellular carcinoma. J Gastroenterol Hepatol 1995;10:674–82.

(91) Coursaget P, Depril N, Chabaud M, Nandi R, Mayelo V, LeCann P, et al. High prevalence of mutations at codon 249 of the p53 gene in hepatocellular carcinomas from Senegal. Br J Cancer 1993;67:1395–7.

(92) Hayward NK, Walker GJ, Graham W, Cooksley E. Hepatocellular carcinoma mutation [letter]. Nature 1991;352:764.

(93) Parkin DM, Muir CS, Whelan SL, Gao YT, Ferley J, Powell J, editors. Cancer incidence in five continents, vol 1. Lyon (France): IARC, 1992.

Notes