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Interspecies comparison of liver carcinogenesis: implications for cancer risk assessment

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The morphology of hepatocellular carcinoma is similar among mice, rats and humans, and the cellular pathogenesis shows features that are both similar and divergent among these species. However, major elements of etiology, molecular pathogenesis, and natural history differ between humans and rodents. As a reflection of these species-determined differences, rodents appear to be neither highly sensitive nor highly specific surrogates for detecting agents that are potential causes of hepatocellular cancer in humans. Results of tests of chemical carcinogenicity in rodents are likely to include a significant number of both false-positive and false-negative risks for humans.

Introduction

Standardized laboratory tests employing mice and rats are used to evaluate the carcinogenic potentials of chemicals, and the results are applied to predict the risk to humans of developing cancer from exposure to the same chemicals. Such an application of animal tests presumes that the process of carcinogenesis in mice and rats resembles that in humans precisely enough to enable these rodents to be reliable surrogates for humans. Comparative analysis of hepatocellular carcinoma (HCC*) may provide a useful test of how closely rodents resemble humans in the development and expression of a site-specific neoplasm. HCC is the most frequent neoplasm elicited in mice and rats exposed to test chemicals; one-third to one-half of all the chemicals that are carcinogenic in rodent tests are hepatocarcinogens (1,2). Worldwide, HCC is also one of the most common visceral neoplasms of humans (3). Since the histopathological features of HCCs are similar in rodents and humans (4–9), these facts may suggest that the development and expression of HCC in these species is closely analogous. Similar patterns of development and expression of HCC in mice, rats, and humans would support the use of rodents as substitutes for identifying risk factors of HCC in humans. In addition to the pathological features of the fully expressed neoplasm, interspecies comparison requires analysis of the following aspects of HCC: (i) the etiology (the causes or risk factors), (ii) the pathogenesis (the cellular and molecular developmental processes), and (iii) the natural history (the pattern of expression during the life of the animal). The extent to which all of these features of hepatocarcinogenesis cohere in all three species determines the similarity of HCC among them. Recent intensive scrutiny of the pathological lesions that precede the appearance of HCC, and of the conditions and agents that entrain the process of hepatocarcinogenesis in rodents and humans, provides the basis to approach an interspecies comparison of HCC. However, significant gaps still prevent a complete and uniform description of etiology, pathogenesis, and natural history of HCC in these species.

***Abbreviations:** HCC, hepatocellular carcinoma; HCAs, hepatocellular adenomas; HBV, hepatitis B virus; FAH, foci of phenotypically altered hepatocytes; HN, hyperplastic nodules; AHN, adenomatous hyperplastic; HCV, hepatitis C virus; WHV, woodchuck hepatitis virus; GSHV, ground squirrel hepatitis virus; AFB, aflatoxins; CPDB, carcinogenic potency database; NTP, National Toxicology Program.

Material and methods

Pathology

Hepatocellular neoplasms, both malignant HCCs and benign hepatocellular adenomas (HCAs), evince similar, histopathological patterns and cytological aberrations in all three species considered here (4–8). The qualitative similarity of histological and cytological features of hepatocellular neoplasms in humans, rats, and mice is reflected by the similar descriptive terms that are applied to them (Table I). In each species, HCCs demonstrate a histological/cytological spectrum from well-differentiated to poorly differentiated. HCCs vary in size from microscopic lesions that contain only a few malignant cells to single massive or multiple small tumor nodules that replace virtually the entire hepatic parenchyma. The structural patterns of well-differentiated, but malignant, HCCs blend insensibly with structural patterns of normal liver parenchyma and of benign HCAs (4–9); in well-differentiated HCC, individual cells may closely resemble normal hepatocytes and form plates (or trabeculae) only two or three cells thick (4–9). Well-differentiated HCC may be composed of hepatocytes containing diploid or near-diploid amounts of DNA (10–13); only the cytological characteristics of malignancy in individual hepatocytes (nuclear atypia and increased nucleus to cytoplasm ratio) and the organization of cells into abnormal tissue patterns define these well-differentiated tumors as malignant. Less well-differentiated HCCs are composed of cytologically more atypical cells, which show markedly abnormal ratios of nuclear to cytoplasmic areas and contain abnormal (aneuploid) amounts of DNA, organized in thick, multicellular trabeculae, solid masses, or gland-like (adenoid or acinar) arrangements (4–9). Even more poorly differentiated HCCs contain cells that are so cytologically bizarre as to cause difficulty in discerning their epithelial origin and in detecting a structural organization resembling hepatic tissue, and include spindle-shaped cells that morphologically resemble mesenchymal cells (6–8). HCCs always distort the normal hepatic structure, displacing, infiltrating, and compressing normal tissues (4–9). Grossly, HCC may be nodular, massive and/or diffuse (6); nodular lesions may be microscopic in size and multiple in number. Poorly differentiated HCCs usually present no problems to the histological determination that they are malignant, but the precise histological distinction between benign HCAs and well-differentiated, but malignant HCCs in both humans and rodents is often difficult (4,5,7,8).

Hepatoblastoma is an uncommon variant of HCC that differs in rodents and humans. In humans, hepatoblastoma is composed of embryonal or fetal hepatocyte-like cells which may also express aberrant epithelial or mesenchymal differentiations, including squamous epithelial (epidermal), cartilaginous, osteoid, myoblastic, and/or neuroendocrine (7,8). Hepatoblastoma originates from embryonal hepatocytic precursor cells (hepatoblasts) during liver development in humans, and its occurrence is virtually limited to children under three years of age, although rarely it occurs in adults (7,8). Cytologically somewhat similar tumors occur occasionally in mice (9,14) and rats (5), but *only* in adult animals and in association with typical HCC (4,5,9,14); murine hepatoblastoma represents highly progressed, poorly differentiated HCC (14).

In both humans and rodents, benign HCAs are composed of neoplastic hepatocytes that closely resemble their normal counterparts (4–9). Individual hepatic plates in HCAs appear to be nearly normal, with minimally increased widths. The most distinctive histopathological feature of this well-differentiated, cytologically benign hepatocellular tumor centers on its failure to ‘fit’ into the normal liver structure (4–9); HCAs lack portal tracts and bile ducts, hepatic plates that cross the boundary between neoplastic and normal tissue are not smoothly continuous, and sinusoids of HCAs are structurally

Table I. Hepatocellular neoplasms in humans and rodents

	Human (6–8)	Rat (5)	Mouse (4,9,14)
Benign neoplasms	Hepatocellular adenoma	Hepatocellular adenoma	Hepatocellular adenoma
Malignant neoplasms	Hepatocellular carcinoma	Hepatocellular carcinoma	Hepatocellular carcinoma
	Trabecular	Trabecular	Trabecular
	Acinar		Solid
	Adenoid (mixed)	Adenocarcinoma (mixed)	Hepatic cell 'adenocarcinoma'
	Fibrolamellar		
	Sclerosing		
	Giant cell	'Sarcoma'	
	Unidifferentiated	Poorly differentiated	
	Hepatoblastoma	Hepatoblastoma	Hepatoblastoma

Table II. Terminology of non-neoplastic (preneoplastic) lesions composed of hepatocytes in humans and rodents

Human (7,8,38–41,47,48,51)	Rat (5,27,32)	Mouse (4,9,27)
Heteromorphic (dysplastic) focus	Altered cellular focus	Altered cellular focus
Hyperplastic nodule	Hyperplastic nodule	Hyperplastic nodule
Usual cirrhotic nodule		
Macroregenerative nodule (Adenomatous hyperplasia)		
Focal nodular hyperplasia		
Nodular regenerative hyperplasia		

abnormal (4–9). Large HCAs, which in humans reach 15 cm or more in diameter (6–8), compress the adjacent normal parenchyma (4–9). In focal areas or occasionally throughout the tumors, neoplastic hepatocytes of HCAs may store excess fat or glycogen and stain aberrantly, either more acidophilic or more basophilic than the surrounding normal hepatocytes (4–9). HCAs also may include a few dysplastic hepatocytes that contain atypical nuclei, and, rarely, frank malignant change (7,8).

Populations of neoplastic hepatocytes are clonal or quasiclonal in the majority of HCAs and HCCs of both rodents (15–17) and humans (18–23); however, only a small number of HCAs have been studied in humans (23). Clonality of hepatic neoplasms has been assessed by analyzing X-linked gene products or genes in both rodents (15,17) and humans (22,23), and by examining the patterns of genomic integration of hepatitis B virus (HBV) DNA in humans (18–21). In rodents, clonality of hepatic neoplasms has also been assessed in the mosaic livers of chimeric animals (16). Since the liver is normally composed of embryonically positioned patches of genetically similar cells, separated by patches of genetically dissimilar cells (24), assessment of clonality by analysis of X-linked gene products and by the use of chimeric animals does not entirely eliminate the possibility that the tumors arise polyclonally from cells located within a single patch composed of genetically similar cells. However, analysis of patterns of genomic integration of HBV DNA provides impeccable evidence of clonality, since each integrant is random (18,19,21).

The tissue setting in which HCC is typically found differs markedly in rodents and humans. Unlike the situation in humans, chronic hepatitis and cirrhosis are uncommon lesions in the livers of rats (25) and mice (26) that develop HCC. In rodents, HCC usually occurs in association with non-neoplastic proliferative lesions composed of phenotypically aberrant hepatocytes, including foci of phenotypically altered hepatocytes (FAH) and hyperplastic nodules (HN) (Table II) (9,27). FAH may be composed of cells that are more 'clear' (more weakly stained), more acidophilic, and/or more basophilic than the surrounding, normal hepatocytes (as well as by combinations of cells that stain in each of these ways) (27). Altered hepatocytes in FAH may store more or less glycogen (27) and iron (27); they may express excess or deficient content/activity of some metabolic enzymes, such as glucose 6-phosphatase, adenosine triphosphatase, γ-glutamyl transpeptidase, and glutathione S-transferase P (27), and of growth factors such as transforming growth factor-α (TGFα) (28,29); and they generally proliferate at abnormally high rates (27). Hepatocytes of HN often embody these and additional abnormal phenotypic properties, such as expression of insulin-like growth factor II (IGFII) (30–32), and they form nodules that disrupt hepatic plates without compressing the surrounding normal parenchyma (4,9,33). Hepatocytes of FAH and HN in rodents are usually diploid (27), occasionally aneuploid (27), and apparently clonal (16,34–37).

HCCs in humans are usually associated with chronic hepatitis (6,8,38,39), and world-wide, 60 to 80% of HCCs in humans occur in cirrhotic livers (6–8,40). Cirrhosis, which is the end result of acute and chronic hepatitis, is characterized by the dissection of the parenchyma into nodular aggregates of hepatocytes by bands (septa) of connective tissue; collagenous connective tissue replaces hepatocytes that have been destroyed during acute and chronic hepatitis (41). The nodular aggregates of hepatocytes in cirrhotic livers, termed regenerating or cirrhotic nodules, vary in size from less than 3 mm to greater than 5 cm in diameter, depending on the cause and chronicity of the cirrhotic process (41). Hepatocytes in cirrhotic nodules frequently show dysplastic changes (42,43) and are often clonal (44,45). Nodular aggregates of abnormal hepatocytes also may occur in non-cirrhotic livers of humans in association with chronic hepatitis and HCC. In Alaskan natives chronically infected with the hepatitis B virus (HBV), HCC usually occurs in the absence of cirrhosis, but accompanied by chronic inflammation and mild fibrosis (46). The livers of these patients frequently contain foci and HN composed of dysplastic hepatocytes that may blend directly into HCC (38).

Large hepatocytic nodules (>8–10 mm in diameter) variously termed adenomatous hyperplastic (AHN) or macroregenerative nodules (MRN) when they are associated with small (possibly early) HCC in cirrhotic livers (47, 48) and hyperplastic nodules when associated with HCC in noncirrhotic livers (38,39), are thought to be the immediate preneoplastic precursors of HCC in humans. The terms and concepts that have been applied to nodular aggregates of hepatocytes in human livers are highly variable, and an international working group has proposed new designations for them (49). The working group categories, which are based only on the histocytological characteristics (but not on phenotypic properties) of cells contained in nodules, include regenerative, dysplastic and neoplastic nodules (49) (Table III). Dysplastic hepatocytes are found in both foci and nodules in cirrhotic and noncirrhotic livers in humans (7,8,42,43), and are associated with elevated risk of HCC (50).

Human livers express certain other non-neoplastic proliferative lesions, including focal nodular hyperplasia and nodular regenerative hyperplasia (7,8,51) (Table III), that do not resemble any of the lesions found in rodents' livers. These lesions do not usually occur in association with HCC in humans, and do not appear to confer increased risk to the development of HCC (7,8,51). However, the hepatocyte populations contained in some focal nodular hyperplasias are clonal or quasiclonal (23), and this lesion is most common in young women, occasionally occurring in association with HCA (51).

Only a few studies in humans have attempted to assess possible phenotypic abnormalities of cells in foci and nodules associated with HCA and HCC (52–66), as has been done in mice and rats (4,27,23,59). FAH expressing cytological or histochemical aberrations have been identified in association with HCC of unknown cause occurring in children (53); in association with HCA in female users of contraceptive steroids (54); and in livers of individuals who have primary hemochromatosis (which is associated with increased risk of HCC) (64,65). A variety of lesions associated with HCC in humans and rodents show similar aberrations in iron storage (53–56,64–66). Evidence that FAH are regularly associated with non-neoplastic and neoplastic nodular lesions is provided by two recent prospective studies (58,63). In a study of 95 consecutive medicolegal autopsies, FAH and HN were identified through the aberrant storage of glycogen and iron in the hepatocytes that composed them in 11.6% of the human livers examined (58). The occurrence of FAH and HN was significantly clustered in livers of patients who consumed >90 g/day of ethanol or who had cirrhosis (type unspecified) (58). FAH were also found in two of five livers that contained hepatocellular nodules of hyperplastic or neoplastic types (focal nodular hyperplasia or HCA) (58).

Table III. Terminology of hepatocellular nodules in humans

A.	Proliferative lesions (49)	
	1. Monoacinar regenerative nodule	
	a. Without collagenous septa	Nodular regenerative hyperplasia (7,8,51)
	b. With collagenous septa	Cirrhotic or regenerative nodule (40,41)
	2. Multiacinar regenerative nodule	
	a. Without collagenous septa	Hyperplastic nodule (38,39)
	b. With collagenous septa	Type I macroregenerative nodule (AHN) (47,48)
	3. Focal nodular hyperplasia	Focal nodular hyperplasia (7,8,51)
B.	Dysplastic lesions (49)	
	1. Dysplastic focus	Heteromorphic (dysplastic) focus (8)
	2. Dysplastic nodule	Type 2 macroregenerative nodule (AHN) (47,48)
	a. Low grade	
	b. High grade	Borderline lesion (48)
C.	Neoplastic lesions (49)	
	1. Hepatocellular adenoma	Hepatocellular adenoma (6–8)
	2. Hepatocellular carcinoma	Hepatocellular carcinoma (6–8)

Analysis of FAH in livers explanted from recipients of liver transplants provides further evidence that FAH composed of hepatocytes that abnormally express glucose 6-phosphatase and glycogen phosphorylase activities, and that store excess glycogen, are common in human livers that are the site of chronic hepatitis, cirrhosis and HCC (63); eight of 16 cases of posthepatitic (HBV or HCV) cirrhosis (50%), 5 of 8 cases of alcoholic cirrhosis (63%), and 14 of 14 cases of HCC (100%) contained foci of phenotypically aberrant hepatocytes. Though limited in number, these studies suggest that focal aggregates of hepatocytes in diseased human livers express phenotypic aberrations that resemble abnormalities of hepatocytes in FAH and HN of rodents.

The different tissue settings in which HCC develops in rodents and humans include the universal occurrence of FAH and HN containing phenotypically aberrant hepatocytes in rodents and the usual occurrence of cirrhosis in humans. These differences may be more apparent than real. In both rodents and humans, hepatocytes in nodular aggregates share several aberrant properties (including frequent dysplasia, apparent clonality, and, possibly, similar types of phenotypic alterations), whether or not they are associated with the fibrotic scarring that defines cirrhosis. Cirrhosis is clearly not essential for the development of HCC in humans. Chronic hepatitis without fibrosis regularly accompanies the occurrence of HCC in some rodent species, including woodchucks (67) and Beechey ground squirrels (68) infected with hepadnaviruses closely related to a major viral risk factor for HCC in humans. FAH and HN, identical phenotypically to those found in mice and rats, also accompany HCC in chronically WHV-infected woodchucks (69). Similarly, classic FAH and HN are found in transgenic mice engineered to express portions of the HBV genome in the liver (70).

Etiology

Numerous chemicals have been tested for their carcinogenic potentials in bioassays performed in mice and rats under standardized laboratory conditions. Standardized carcinogenicity assays in rodents (71) include control groups exposed to vehicle but not agent, as well as groups exposed to the test agent at various doses, including at least the highest dose that is tolerated (maximum tolerated dose or MTD) by the test animal without incurring non-neoplastic tissue injury or growth failure, and a dose that is approximately one-half the MTD. Animals of both control and test groups are examined for neoplasms during their lifetimes. In contrast to the direct manner in which carcinogenicity is evaluated in laboratory animals, the carcinogenic potential of agents for humans must be inferred from epidemiologic studies that seek to determine prospectively the tumorigenic outcomes in groups of people that are either exposed or not exposed to an agent or condition (cohort study) (72). Alternatively, groups of people in which a particular tumor has been diagnosed are compared retrospectively to control groups in which this tumor has not occurred (case-control study), and the manner in which these two groups have differed in terms of exposure to agents or conditions that may be carcinogenic is sought (72). Individuals in both index and control groups must be matched closely by age and sex, and often by various conditions of lifestyle and environment as well. Retrospective determination of exposure of individual humans is usually a challenge, as is the identification of conditions that may confound interpretation of the results obtained. In general, situations in which humans are exposed to chemicals, are characterized by low exposure dose and by exposure to multiple agents simultaneously, in contrast to laboratory studies employing rodents. Consequently, epidemiologic studies in humans may have low sensitivity and specificity for identifying specific causes of cancer.

The major human hepatocarcinogens that have been identified are the hepatotropic viruses, HBV and hepatitis C virus (HCV) (73) (Table IV). Infection with HBV and HCV is signaled by the presence of antibodies to components of these viruses in the plasma and/or by viral nucleic acids in the plasma or tissue of patients that are, or have been, infected (and, in the instance of HBV, by the incorporation of fragments of the HBV genome into the genomes of hepatocytes), providing sensitive and highly specific measures of viral exposures. Chronic infection with either HBV or HCV greatly increases the risk of HCC in humans (100- to 200-fold risks are found in epidemiological studies), as compared to humans who are not infected (73). The hepatocarcinogenicity of HBV in humans is supported by studies of the effects of experimental infection of woodchucks and Beechey ground squirrels with hepadnaviruses that are closely related to HBV. Chronic infection of woodchucks with the woodchuck hepatitis virus (WHV) (67) and of Beechey ground squirrels with ground squirrel hepatitis virus (GSHV) (68) are associated with the occurrence of HCC in these animals. Mice and rats are not known to be susceptible to a species-specific hepadnavirus.

Dietary consumption of aflatoxins (AFB) in food grains contaminated with *Aspergillus flavus* and related fungi is also a major cause of HCC in humans (74). Although population-based epidemiological studies suggest that the average level of AFB in diets consumed by humans is positively correlated with the incidence of HCC in a human population (75), interpretation of these studies has been hampered by the inability to assess exposure of individuals in the population groups studied. Urinary excretion of the excised promutagenic AFB–*N*7 guanine DNA adduct, which specifically reflects AFB-damage to DNA, can be measured precisely, providing a sensitive and specific marker of current aflatoxin exposure (76). Molecular epidemiological assessment of AFB exposure of individuals by quantifying urinary AFB–*N*7 guanine excretion confirms an ~8-fold increase in risk of HCC in humans exposed to AFB (76,77).

As of July 1996, the International Agency for Research on Cancer (IARC) had identified 58 chemicals, complex chemical mixtures, industrial processes, and therapeutic agents for which they conclude there to be sufficient evidence for carcinogenicity at some site in humans (78,79); five of 35 chemicals or therapeutic drugs (14%), two of 10 complex chemical mixtures (20%), and one of 13 industrial processes (8%) that are human carcinogens are associated with HCC in humans. In addition to HBV, HCV and AFB, risk factors for development of HCC in humans (Table IV) include exposure to high levels of vinyl chloride monomer in the workplace (78), use of therapeutic drugs containing certain contraceptive or anabolic steroids (78), and consumption of ethanol-containing beverages (78,80). Additional agents that are occasionally associated with HCC in humans include ingestion of inorganic arsenic compounds (81) and administration of compounds that emit internal radiation, as occurred after medical usage of colloidal thorium dioxide (Thoratrast) for the radiological imaging of blood vessels (82,83). Smoking of tobacco has also been suggested by some (84–88), but not all (89–91), epidemiological studies to increase the risk of HCC. The latter studies indicate the difficulty to determine the hepatocarcinogenic actions of potentially weak carcinogenic agents in the presence of much more potent agents, such as HBV and HCV infection. Great care is required to prevent the confounding of epidemiological identification of potentially weak hepatocarcinogens. Several epidemiological studies designed to detect the involvement of specific types of environmental and industrial chemicals (including pesticides, herbicides and organic solvents) in causation of HCC and hepatoblastoma in humans, have variously yielded negative, equivocal and weakly positive results (92–96). For example, an

Table IV. Comparison of etiologic risk factors for hepatocellular carcinoma in humans with risk factors for rodents

Human (73,74,78–88,98–107)	Rat (1,2,123–127)	Mouse (1,2,123,129,130)
Infectious agents		
Hepatitis B virus	−	−
Hepatitis C virus	−	−
−	−	*Helicobacter* spp.
Environmental/industrial chemicals		
Aflatoxin B1	+	−
Inorganic arsenicals	−	−
2-Naphthylamine	−	+
Vinyl chloride	+	+
−	Numerous other chemicals	Numerous other chemicals
Therapeutic drugs		
Contraceptive steroid mixtures	(Ethinyl estradiol)	−
Anabolic steroids	−	−
(Azathropine)		
−	Cyclophosphamide	?
−	Phenacetin analgesics	?
−	−	Nonsteroidal estrogens (diethylstilbestrol)
−	Tamoxifen	?
Metabolic liver diseases		
Primary hemachromatosis	−	−
Tyrosinemia, type 1	−	Lethal albino syndrome
Glycogen storage disease, type 1	−	−
Porphyria cutanea tarda	−	−
Acute intermittent porphyria	−	−
α1-antitrypsin deficiency	−	−
Wilson's disease	Chronic hepatitis in Long-Evans Cinnamon rats	−
Personal habits		
(Cigarette smoking)	−	−
Ethanol drinking	+ promoter	−

elevated risk of HCC in Vietnamese farmers was attributed to pesticide/herbicide exposures (96); farmers who recalled using >30 litres of organochlorine or organophosphorus pesticides each year from 1960 to 1972, and, also, resided during that interval in areas of South Vietnam sprayed with Agent Orange, showed a modestly elevated risk to HCC (96). This study found that chronic infection with HBV and ethanol consumption were the strongest risk factors for HCC in the Vietnamese farmers, but the farmers were not evaluated for other plausible strong risk factors for HCC, such as chronic infection with HCV and exposure to AFB (96). Furthermore, HCC does not occur at increased incidence in US soldiers who were exposed to Agent Orange in Vietnam (97).

Risk of developing HCC in humans is associated with several genetically determined metabolic abnormalities that may be inherited (98–107) (Table IV). Most of these genetic states of increased susceptibility to HCC in humans are characterized by inborn errors of metabolism that lead to the accumulation of metabolic products in hepatocytes. Liver damage resulting from direct or indirect toxic effects of the abnormal accumulation of metabolic products leads to chronic hepatitis, cirrhosis, and HCC. Defective DNA repair may be the basis of increased risk of HCC in humans with ataxia telangiectasia (103,104). Nevertheless, of the several other well-studied syndromic types of genetic defects in DNA repair (108), none are known to be associated with increased risk of HCC. Genetic polymorphisms in hepatic drug metabolizing/catabolizing enzymes may also influence risk to HCC in some families, as has been demonstrated by increased risk associated with impaired ability to catabolize activated AFB metabolites (109–112). Hereditary susceptibility to HCC in humans in the absence of overt genetic metabolic diseases has been posited on the basis of tumor clusters in families (113,114), but such familial clusters of HCC could result from common exposures of family members to environmental risk factors, such as HBV infection.

The etiology of HCC in humans is clearly multifactorial (115). Epidemiological evidence suggests that concurrent chronic infections with HBV and HCV (73,116,117) and exposure to AFB (74), ethanol abuse (80,118–121), and possibly tobacco smoking (84–88), may amplify the risk to development of HCC. In addition, these agents can interact with other known risk factors, including genetic abnormalities that are associated with increased risk of HCC, to increase risk further in humans (122). Environmental chemicals may also be involved in the multifactorial etiology of HCC in humans even though their contribution to overall risk cannot be readily separated in epidemiological studies from the much higher risks posed by chronic infection with HBV and HCV. However, HCC occurs at highest incidence in human populations of predominantly nonindustrialized countries in which chronic infections with HBV and/or HCV are epidemic (73) and where environmental pollutants may not be significant; furthermore, in Japan, a highly industrialized country in which the incidence of HCC is high and rapidly escalating, the major causes are also HBV and HCV infection (120,121).

In contrast to the situation in humans, the major hepatocarcinogens identified in mice and rats are chemicals. The Carcinogenic Potency Database (CPDB), which includes data from the US National Toxicology Program (NTP) (2) and other published studies, contains results of tests on 1230 chemicals conducted in laboratory animals (123). Of 1052 chemicals reviewed in 1991, 533 (50%) are carcinogenic at some site in at least one rodent species (1). The liver is the most frequent site affected by cancer in carcinogenicity tests in both mice and rats: of 299 CPDB-listed chemicals that are carcinogenic in mice, 171 (57%) affect the liver, whereas of 354 chemicals that are carcinogenic in rats, 143 (40%) produce liver tumors. The liver is the most frequent site in rodents affected by cancer from exposure to either mutagenic or nonmutagenic chemicals (124). It is striking that, except for one recently identified agent, bacterial and viral agents are not known to be hepatocarcinogenic in rats and mice. The exception is chronic hepatic infection of mice with bacteria of the *Helicobacter* species, which is associated with increased risk to HCC (125). Although infection of the stomach by *H.pylori* is associated with gastric cancer in humans (126), hepatic infection with *Helicobacter* is apparently not known in humans.

Rodent strains are affected by a few genetically predicated metabolic diseases and by other genetic conditions that increase the risk of HCC (Table IV). Long-Evans Cinnamon rats with hereditary chronic hepatitis (127) have increased risk of HCC associated with the genetic lack of a copper-carrying adenosine triphosphatase (128), a condition that is genetically and molecularly identical to Wilson's disease in humans (100,129). Lethal albino mice suffer from a genetic defect in the enzyme fumaryl acetoacetate hydrolase, which leads to accumulation of toxic compounds in hepatocytes and increased risk of HCC (130). This disease in mice is identical to type 1 tyrosinemia in humans (102). The inbred mouse strains C3H and CBA, and strains and crosses derived from them (such as B6C3F1), spontaneously develop HCA and HCC at high incidence, and they are also more susceptible to development of liver tumors after exposure to carcinogenic chemicals than are mice of other strains (131–133). The genes that determine increased susceptibility to HCA and HCC in mice are distributed among several chromosomal sites

Table V. Chromosomes involved in hepatocellular carcinoma in rodents

Mouse			Rat
Susceptibility loci (134–137)	Structural abnormalities (357)	Loss of heterozygosity (352,355,365)	Structural abnormalities (358–360)
	1	1	1
2		2	2
			3
4	4		4
5		5	
		6	6
7	7	7	7
8		8	8
9		9	
10			10
			11
12	12	12	
		13	
		14	
		18	
19	19		

(Table V) (134–136). The actions of these loci appear to differ from the other metabolic abnormalities associated with increased risk of HCC in humans and rodents. Murine susceptibility loci may act by allowing more rapid proliferation of hepatocytes in FAH and HN (134). Recently, two loci that confer resistance to HCC in mice have also been identified (137); these loci seem to act by blocking the activity of some of the previously identified susceptibility loci (134).

The contrasting nature of the major hepatocarcinogens for rodents and humans suggests that the results of carcinogenicity tests performed in mice and rats may not accurately predict whether humans will respond in a similar manner to exposure to a specific chemical. Several authors have approached the assessment of the utility of carcinogenicity studies performed in mice and rats to predict risk in humans by analyzing the accuracy with which the results of carcinogenicity tests obtained in one of these two rodent species can be used to assess risk in the other (138–140). These analyses in mice and rats can be made from data obtained by tests of carcinogenicity that are performed under nearly identical conditions in each rodent species. Concordance analysis of results of carcinogenicity tests in rodents shows that the identification of a carcinogen in either mice or rats *at any site* is only a fair predictor that this chemical will cause cancer *at any site* in the other species (138–140), with sensitivities and specificities for predicting the outcome in the other species of ~75%. When limited to liver tumors, the predictivity for one rodent species of results of carcinogenicity tests obtained in the other species is even less accurate than for tumors at any site. Of the 131 chemicals on the CPDB list that cause liver tumors in mice, only 85 (65%) also cause tumors of the livers in rats, and of 79 chemicals that affect the livers of rats, 70 (89%) also cause liver cancer in mice (1). Since each rodent species is affected by several carcinogenic chemicals to which the other species is not susceptible, extrapolation of risk from either mice or rats to the other rodent species includes a significant number of both false-positive and false-negative assignments of risk.

One might suspect intuitively that predicting from a rodent species to humans will be less accurate than will predicting from rodent to rodent. The database for directly testing this assumption by comparing the liver site concordance among humans, mice, and rats after chemical exposure is extremely small (140). Of the 58 agents or processes that the IARC has identified as carcinogenic for humans (76,79) most are also carcinogenic at some site in a rodent of at least one species and sex. Fifteen of the human carcinogenic agents (26%) are associated with HCC in at least one of the species human, rat, and mouse (Table VI). The human liver is affected by 9 of 17 agents (53%), whereas the rat liver is the site of tumor formation for 8 of 16 (50%), and the mouse liver for 6 of 10 (60%) of the agents which have been appropriately examined in these rodent species (74,78,79,141). Despite the fact that the liver is affected by approximately one-half of the agents in all three species, only 1 of 10 informative instances (10%) shows concordance of liver site in all three species. Concordance of liver site in humans and rats occurs in only 4 of 16 (25%) informative pairings, in humans and mice in only 2 of 10 (20%), and in rats and mice in 4 of 10 (40%).

Analysis of the predictivity of both positive and negative results by comparing responses in either mice or rats to humans cannot be done, since it is impossible unambiguously to identify specific chemicals that are *not* carcinogenic in humans. Nevertheless, an attempt to designate possible human noncarcinogens and to assess the concordance of tests in humans and rodents has been made by analyzing 29 chemicals for which IARC has found inadequate evidence (for whatever reason) for carcinogenicity in humans (142). Although human noncarcinogenicity of an agent cannot be proved, several negative epidemiologic studies in humans were available for 13 chemicals that had also been tested in rodents, only two of which failed to produce tumors in at least one rodent species and sex (142). Of interest is the observation that the liver is frequently the site of cancer induced by these chemicals in the rodent studies. As noted above for IARC human carcinogens, there was poor concordance of liver site among species. The authors concluded that results of rodent tests *may* include many false-positive indications of human risk (142).

Comparative analysis of the carcinogenicity of pharmaceutical agents in humans and rodents is of interest because humans may be chronically exposed to therapeutic drugs in known doses that are similar to the high doses to which rodents are exposed in carcinogenicity tests. Of 183 pharmaceutical agents analyzed by IARC for their carcinogenic risk to humans (79,143), 20 are conclusive or probable carcinogens for humans (Groups 1 and 2A, respectively, in the IARC terminology), and an organ site is known both for humans and rodents (Table VI). Three drugs (combined oral contraceptive steroid combinations, azathioprine, and anabolic steroids) are considered by IARC to be associated with risk of HCC in humans. Contraceptive steroid formulations have not been tested for carcinogenicity in rodents, but ethinyl estradiol, a component of some contraceptive mixtures, promotes HCC in rats (144); azathioprine is not hepatocarcinogenic in mice and has not been tested in rats (143); the androgenic steroid, testosterone is hepatocarcinogenic for rats but not mice (143). Of the 17 other pharmaceutical agents that are conclusive or probable human carcinogens but do not cause HCC in humans, 14 have been studied in rats (79,143) and 15 have been studied in mice (143). In rodent tests, HCC occurs only in rats exposed to diethylstilbestrol (143), phenacetin (143), and tamoxifen (141) and in mice exposed to cyclophosphamide (143). HCC is an infrequent neoplasm in all three species, occurring in ~15% of the tumors associated with drugs in humans and rats, and in ~6% of the drug-related tumors in mice. Concordance of liver site among these three species is lacking, save for humans receiving some combined formulations of contraceptive steroids and rats exposed to ethinyl estradiol.

Although somewhat limited in extent, these data suggest that the susceptibility of humans, mice and rats to the hepatocarcinogenicity of specific chemicals may differ. Four chemicals that diverge widely in their hepatocarcinogenicity for humans and rodents, phenobarbital, ethanol, vinyl chloride and AFB, illustrate striking species-dependent variations. Phenobarbital induces HCC in susceptible strains of mice and is a strong promoter of HCC both in resistant strains of mice and in rats (145–146). Despite its potency in inducing or promoting HCC in mice and rats, it now seems certain that phenobarbital ingestion is not associated with increased risk of HCC in humans, although the IARC designates phenobarbital as a possible human carcinogen on the basis of rodent tests (Group 2B) (143). In three large cohort epidemiological studies in England (147), the US (148), and Denmark (149), a combined population of >8000 patients who received long-term treatment with phenobarbital did not show evidence of an elevated incidence of HCC when the Denmark studies were corrected for documented exposures of patients to the strong human hepatocarcinogen, Thoratrast (82,83). Consumption of alcoholic beverages (ethanol) is classified by the IARC as a human carcinogen, with the liver being a major site (80). Ethanol appears to potentiate the hepatocarcinogenicity of chronic infections with both HBV and HCV in humans (118,119). Ethanol is not considered by the IARC to be a rodent carcinogen (80), although it enhances (or promotes) HCC development in rats (150–152) and is designated as a hepatocarcinogen for rats in the most recent CPDB list (123). Both phenobarbital and ethanol may act mainly as promoters of HCC development in one or more species, but the mechanisms of their actions are incompletely elucidated and the basis for their species-different effects is unknown.

Differences in metabolic properties of hepatocytes among rodents and humans appear to predicate species-dependent susceptibility to HCC from vinyl chloride and AFB. HCCs in rats exposed to vinyl chloride contain a low frequency of AT to TA transversion mutations in the second base of codon 61 of the H-*ras* gene (153), but in humans exposed to vinyl chloride, GC to AT transversion mutations in the second base of codon 13 of the K-*ras* gene are found in angiosarcomas (154), but not in HCC. These results suggest that even when humans and rodents are exposed to the same chemical mutagen-carcinogen, the specific mutations and cells or tissues affected may differ among species. The differential species-related susceptibility to the hepatocarcinogenic effects of AFB (mice are resistant; rats and humans are susceptible (1,2,74)) reflects differences among these species in the metabolic activation of AFB to the highly reactive AFB 8,9-epoxide, which forms

Table VI. Concordance of liver site among humans, rats, and mice for IARC-designated human carcinogens

Carcinogenic chemical	Sites		
	Human (74,78–80,143)	Rat (1,2,141,143,144)	Mouse (1,2,143)
Aflatoxins	LIVER	LIVER, kidney, colon	Lung
4-Aminobiphenyl	Bladder	Mammary gland, intestines	LIVER, bladder
Arsenic compounds	LIVER, skin, lung, kidney, intestines, bone marrow	Negative	Negative
Auramine	Bladder	LIVER	LIVER
Azathioprine	Lymphatic tissue, skin (LIVER)	Lymphatic tissue, Zymbal gland	Lymphatic tissue
Benzidine	Bladder	Mammary gland, Zymbal gland	LIVER
Cyclophosphamide	Bladder, bone marrow	LIVER, bladder, testis, lung, mammary gland, lymphatic tissue	–
Ethanol	LIVER, oral cavity, pharynx, larynx, esophagus	LIVER	–
2-Naphthylamine	LIVER, bladder	Bladder	LIVER, lung
Phenacetin analgesics	Renal pelvis, ureter, bladder	LIVER, kidney, renal pelvis	–
Steroids, androgenic (testosterone)	LIVER	Cervix, uterus	Prostate
Steroids, contraceptive mixtures	LIVER	LIVER, ethinyl estradiol	–
Nonsteroidal estrogens (diethylstilbestrol)	Vagina, cervix, testis, mammary gland, endometrium	Vagina, cervix, mammary gland	LIVER, pituitary, mammary gland
Tamoxifen	Endometrium	LIVER	–
Tobacco smoke	Lung, bladder, oral cavity, larynx, pharynx, esophagus, pancreas (LIVER)	Respiratory tract	–
Vinyl chloride	LIVER, lung, brain, lymphatic tissue, bone marrow	LIVER, Zymbal gland	LIVER, mammary gland

procarcinogenic adducts in DNA, and in the deactivation of the epoxide by conjugation to glutathione by glutathione S-transferase isoforms (M1 isoform in humans; A2 isoform in mice) and/or conversion of the epoxide to nontoxic AFB 1,2-dihydrodiol by epoxide hydrolase (109,110). Species differences in the carcinogenicity of AFB for mice and rats correlate with the much higher activity in mice than rats of the glutathione S-transferase isoform that conjugates the active AFB 8,9-epoxide. Subcellular enzymatic preparations made from liver tissue of mice conjugate AFB 8,9-epoxide at rates nearly 9-fold greater than do enzyme preparations from liver tissue of rats or humans (109,112). These species-related differences in metabolism correlate well with the relative levels of AFB adducts produced in DNA of mice and rats and in albumin of mice, rats, and humans by comparable exposures to AFB (109–111). Genetic polymorphisms that affect the activities of glutathione S-transferase M1 and epoxide hydrolase also affect human subpopulations (110,112). Molecular epidemiologic studies show that a genetic polymorphism causing decreased activity of epoxide hydrolase is associated with a higher risk of AFB-induced DNA damage and HCC in a human population exposed to AFB (as indicated by AFB-induced adducts in DNA) (112). Interestingly, the increased risk of HCC from the epoxide hydrolase polymorphism appears to affect only those humans who are also chronically infected with HBV (112), suggesting a causal interaction of these agents.

Pathogenesis

Pathogenesis is analyzed at both cellular and molecular levels; fundamental pathogenic aberrations at the molecular level are expressed as cells that are phenotypically abnormal and proliferate to form multicellular lesions (foci, hyperplastic nodules, adenomas, carcinomas) composed of cells that are aberrantly differentiated. The essence of cellular pathogenesis of HCC is the progressive dysregulation of differentiation, proliferation, and death of individual and small groups of hepatocytes. Even though the molecular changes that dysregulate hepatocellular proliferation and differentiation may be quite diverse, the altered cells form a limited and reproducible group of cytologic and histologic lesions. The molecular aberrations involved in hepatocarcinogenesis include abnormalities in numerous elements of the signal transduction pathways, and in nuclear transcription factors on which the cell surface-to-nucleus signals converge to affect the expression of specific genes that regulate hepatocyte differentiation, proliferation, and death. Molecular aberrations may also involve the direct alteration of gene structure by mutation of nucleotide sequences or deletion and/or rearrangement of chromosomal segments containing relevant genes. Genes may also be silenced by hypermethylation of some cytosine residues by an epigenetic process. In all three species, understanding of pathogenesis is still incomplete and rapidly evolving.

Cellular pathogenesis

The cellular pathogenesis of HCC is most detailed in rats and mice, in which the progressive proliferation of phenotypically altered hepatocytes leads to the formation of non-neoplastic cellular lesions preceding the appearance of cancer (4,5,9,27,33,155,156). Hepatocarcinogenesis is a sequential, multistep cellular process in rodents, with stages termed initiation, promotion, and progression which can be delineated experimentally (4,5,9,27,33,155,156). Initiation by mutagens-carcinogens involves the formation of a permanent, heritable, aberration in the genome of individual hepatocytes. During the promotion stage, the initiated cells are clonally expanded by consecutive cycles of proliferation. Further genetic changes and focal population expansions by clonal proliferation take place during the progression stage, leading to the emergence of cell progeny which have gained the ability to invade and metastasize. In rodents a few hepatocytes initially express aberrant phenotypic properties that differ from those of the surrounding, unaffected cells and allow affected cells to proliferate differentially (4,5,9,27,33,155,156). Initiated hepatocytes are thought to express enzymes and metabolic products aberrantly as a reflection of altered gene regulation induced by mutagens-carcinogens. Proliferation of the progeny of initiated, phenotypically altered hepatocytes is considered to lead progressively to the formation of FAH and HN, in some of which cytologically neoplastic cells develop and proliferate further to form HCA and/or HCC. FAH, HN and HCA are thought to be the sites in which progressive dysregulation of cellular proliferation and differentiation occurs and irreversible genomic lesions develop (Figure 1). Although the evidence for the occurrence of this cellular sequence of developmental steps and stages of HCC development in rodents is indirect, the phenotypic similarity and the apparent monoclonality of the aberrant hepatocytes that make up both preneoplastic lesions and neoplasms in mice and rats support the lineal progression from FAH to HN, HCA, and/or HCC. The identification of cellular atypia and frank HCC within preneoplastic lesions and HCA (advanced lesions within precursor lesion) correlated with evidence of clonal variants in the advanced lesions, further strengthens the reality of this postulated cellular pathway (4,5,9,27,33,155,156). However, many of the cells in early lesions (FAH and HN) may redifferentiate to a normal hepatocyte phenotype (156) and/or they may die (157), leading to their disappearance. Experimental studies have shown that thousands of FAH precede each HCC that develops in most carcinogenesis models in rodents (156).

An alternate cellular pathway to the development of HCC in rodents involves the proliferation of hepatocyte progenitor cells, termed oval cells, which then may differentiate into normal hepatocytes or into cells of FAH, HN, HCA, and/or HCC (158) (Figure 1). Oval cell proliferation is a prominent feature of experimental hepatocarcinogenesis in rodents, occurring in rats on several experimental regimens (158) and in woodchucks chronically infected with WHV (159). This pathway of hepatocarcinogenesis is supported by studies showing that stem-like cells, clonally isolated from normal rat liver, can give rise when neoplastically transformed to multiple types of hepatic tumors including HCC, hepatoblastomas, cholangiocarcinomas, and 'sarcomas' (160). It seems possible that the development of HCC in rodents may begin

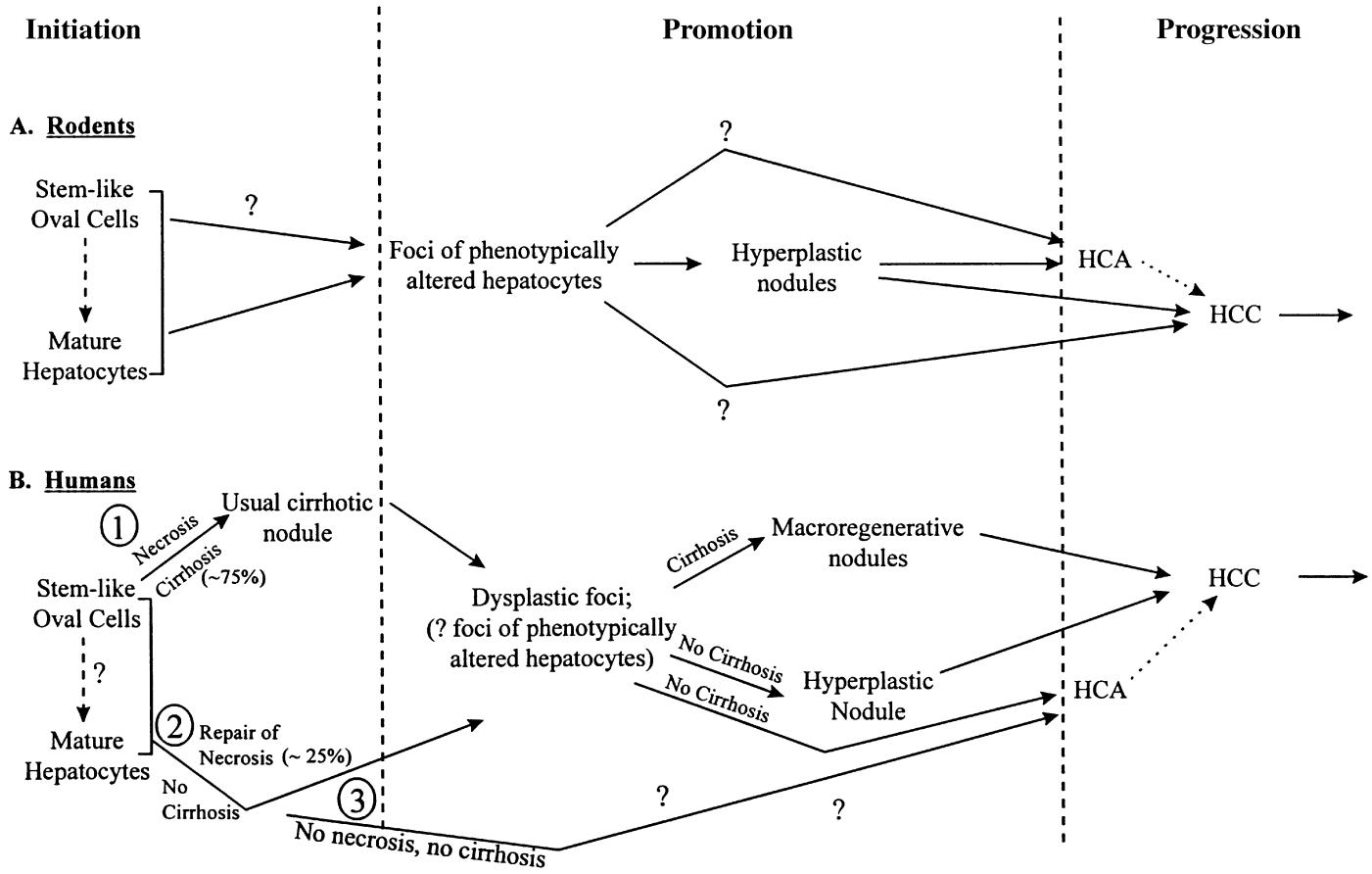

Fig. 1. (**A**) Pathway of cellular pathogenesis of HCC in rodents. The pathway is thought to lead from foci of phenotypically altered hepatocytes, some of which contain initiating lesions, through hyperplastic nodules and HCA to HCC. The cell initially affected by genomic damage that initiates the carcinogenic process may occur in hepatocytes, liver stem cells, or both. Some features of this cellular pathway may be less well-defined in mice; hyperplastic nodules are often indistinct in mice and it is often difficult to distinguish HCA and HCC in mice. (**B**) Pathways of cellular pathogenesis of HCC in humans. A single pathway does not fit all HCC in humans. Pathway 1 is the most frequent, occurring in ~75% of all HCCs. Chronic hepatitis and cirrhosis precede the development of HCC, which appears normally to develop in rapidly expanding cirrhotic nodules, termed macroregenerative or adenomatous hyperplastic nodules. Progression of HCC leads from early intrahepatic lesions to advanced metastases. Pathway 2 occurs in ~25% of HCC in humans. Cirrhosis does not occur, but chronic hepatitis and focal parenchymal hyperplasia are usually prominent features preceding the development of HCC. Pathway 3 is a minor pathway that probably represents much less than 1% of HCC in humans. The lesions that precede HCA in this pathway are largely unknown. The risk of progression from HCA to HCC is very small and HCA may regress by unknown mechanisms.

in either mature hepatocytes or stem-like cells under different circumstances (158,159).

Delineation of the sequence of preneoplastic cellular changes and characterization of the multicellular lesions that precede the emergence of HCC is less complete in humans than in rodents, because it is more difficult to determine when the process of hepatocarcinogenesis is 'initiated' by natural disease in humans than by experimental manipulation in laboratory rodents. Identification of HBV and HCV as etiologic agents for HCC, together with the availability of sensitive and specific methods to detect the effects of these viruses on hepatocytes, has made it possible to begin to define the cellular pathogenesis of HCC in humans (Figure 1). Chronic hepatitis and cirrhosis are relatively frequent and lasting consequences of HBV or HCV infection, and these diseases are the settings in which HCC most often occurs in humans. Preneoplastic cellular changes have been sought most intensively in cirrhotic livers, since most HCC in humans occurs in this setting. Analysis of preneoplastic changes in human livers has emphasized classic morphologic changes of cellular dysplasia (8,42,43); presence of dysplastic hepatocytes in cirrhotic nodules is correlated with increased risk of HCC in prospective studies (50). The larger cirrhotic nodules (multiacinar nodules, previously called AHN or MRN) are an important site of HCC development in human cirrhotic livers (47–49). HCCs appear to emerge in populations of aberrant, but non-neoplastic, hepatocytes contained in cirrhotic nodules or multiacinar nodules through the clonal proliferation of new populations that express additional aberrations, eventuating ultimately in frank neoplasia (19–22). HN containing aggregates of dysplastic hepatocytes, similar to cirrhotic nodules, but not embedded in collagen, are also thought to be the site of cell proliferation and HCC development in noncirrhotic livers affected by chronic hepatitis (12,19,38,39). These nodular lesions, in which HCC frequently develops in humans, resemble HN of rodents in terms of aberrant expression of metabolic enzymes and growth factors, to the limited extent that these functional properties have been studied in humans (52–66). Occurrence of smaller aggregates of phenotypically aberrant hepatocytes in human livers, analogous to FAH in rodents, is less well documented. However, a few studies have identified foci of phenotypically aberrant hepatocytes, in both cirrhotic and noncirrhotic livers of humans (8,58–66), suggesting that preneoplastic cellular lesions in both humans and rodents are similar.

The cellular elements of an alternative pathway to the development of HCC in humans involving the proliferation and differentiation of stem-like ('oval') cells have been described (161,162). As in rodents, it is possible that potential cellular pathways to development of HCC in humans may involve either differentiated hepatocytes or stem-like cells.

The patterns of dysregulated cell proliferation and death are similar during hepatocarcinogenesis in rodents and humans. The major differences among these species include the close association of HCCs with chronic hepatitis and cirrhosis in humans (38–40), both of which are correlated with significant levels of hepatocellular necrosis. Chronic hepatitis, and cirrhosis are typically lacking in livers of mice and uncommon in livers of rats in which HCC develops (26,27). Hepatocellular necrosis resulting from chemical cytotoxicity appears to be neither a reproducible nor a necessary feature of the pathogenesis of HCC in mice and rats in the NTP studies (163,164). Nevertheless, elevated hepatocyte proliferation and death are common features of phenotypically altered hepatocytes in livers of both humans and rodents during hepatocarcinogenesis. Dysregulation of hepatocyte proliferation and death are common features associated with development of HCC in both humans and rodents.

Molecular pathogenesis

The molecular pathogenesis of HCC in rodents and in humans is still incompletely known, although many alterations involving expression of growth factors/receptors, proto-oncogenes, and tumor suppressor genes have been found at various stages of hepatocarcinogenesis. This brief presentation emphasizes the comparison in humans and rodents of alterations in expression of the growth factors TGFα, TGFβ and IGF-II (and their receptors); of constitutive expression and mutation of *myc* and *ras* proto-oncogenes; and of mutation and deletion of the *p53* tumor suppressor gene. A defined sequence of molecular aberrations leading progressively to HCC has not yet been identified in either rodents or humans, but likely molecular pathways are emerging. Because of space limitations, aberrations in many of the molecular elements that regulate the hepatocyte proliferative cycle (165) are not discussed in this review.

Among the earliest alterations in the molecular regulation of proliferation of preneoplastic hepatocytes in both rodents and humans is the acquired expression (or re-expression) of two positive growth factors, TGFα and IGF-II. TGFα is expressed during reparative proliferation of hepatocytes following liver injury in adult animals (166). TGFα is expressed focally in ~10% of FAH of rats exposed to the hepatocarcinogen diethylnitrosamine (DEN) (28,29,167), and more uniformly in a larger fraction of HN and HCC (28,29); TGFα expression may indicate those cells that are progressing from FAH to HCC (29,168). The cognate receptor for TGFα, epidermal growth factor receptor (EGFR), is abundantly expressed on normal, preneoplastic and neoplastic hepatocytes, providing the pathway for a growth-stimulatory autocrine cycle (166,169). Transgenic mice, engineered to express TGFα constitutively in the liver, are more susceptible to development of spontaneous or chemically induced HCC than are their nontransgenic counterparts (170). However, expression of TGFα does not occur during the development of preneoplastic lesions and HCC in rats exposed to some peroxisome proliferators (167), indicating the existence of alternate pathways to HCC. Furthermore, mice in which the TGFα gene has been deleted by targeted disruption continue to develop DEN-induced HCC with somewhat slowed growth, showing that this growth factor is not required for HCC development (171).

TGFα is focally expressed in human hepatocytes in livers that are the site of chronic hepatitis and cirrhosis (172–174), expression occurring most intensely in dysplastic hepatocytes that are infected with HBV (175). In humans, neoplastic hepatocytes in many HCC express TGFα (173,174,176); expression is highest and most uniform in cells of well-differentiated HCC, while cells of poorly differentiated tumors are often negative (176). These observations suggest that elevated and constitutive expression of TGFα is an early and somewhat transient event in the process of hepatocarcinogenesis in humans. Nevertheless, urinary excretion of TGFα in humans provides a marker for the presence of HCC that is about as sensitive as is α-fetoprotein (177–179).

Although not normally expressed in the livers of animals postnatally (30), IGF-II is markedly up-regulated in some HN and most HCC of rats (30,180), in HCC of SV40Tag transgenic mice (32,181), and in HN and HCC of WHV-infected woodchucks (32,181). Expression of IGF-II does not occur in FAH in either rodent species (30–32), is focal in the cells of HN, and is most intense and uniform in HCC (30–32). These observations suggest that the reactivation of IGF-II expression occurs later in the process of hepatocarcinogenesis than does elevated expression of TGFα. IGF-II availability may also be affected by the expression level of the mannose 6-phosphate/IGF-II receptor (M6P/IGF-IIR), which is involved in lysosomal breakdown of this growth factor and is normally expressed ubiquitously (182). M6P/IGF-IIR expression is reduced in a subset of FAH and HN in rats exposed to the hepatocarcinogen DEN (182), but not in livers of rats exposed to peroxisome proliferators (183). In human livers, IGF-II expression occurs in some cirrhotic nodules (184–190) and most HCC, but not in chronic hepatitis (184,186), or in normal liver (187). IGF-II expression is associated with hepatocytes infected with HBV (185,187,188). The plasma level of IGF-II in patients with HCC may be either elevated (189) or not (190); elevated serum levels may contain abnormally processed proIGF-II (189). IGF-II and IGF binding protein II are also overexpressed in hepatoblastoma (191). Increased expression of IGF-II in hepatoblastoma may occasionally result from loss of imprinting (192,193), but more often from usage of an alternate promoter (193,194). Furthermore, increased levels of IGF-II may also result from decreased breakdown as a result of reduction in the level of M6P/IGF-IIR expression. Expression of M6P/IGF-IIR is markedly reduced in HCA and HCC of humans (195). Reduced expression of M6P/IGF-IIR is caused by allelic deletion, resulting in loss of heterozygosity (LOH) (196), which may be coupled with mutation of the remaining allele (197). Imprinting of the M6P/IGF-IIR gene causes hepatocytes of rodents to be highly vulnerable to LOH (196). Decreased expression of M6P/IGF-IIR increases the availability of IGF-II by impairing its degradation. Reactivation of the expression of IGF-II in hepatocytes that express IGF-IR produces an additional growth-stimulatory autocrine cycle. Transgenic mice that overexpress IGF-II in the liver show a somewhat higher incidence of spontaneous HCC in males (but not in females) (197).

TGFβ1 is excreted as inactive preproTGFβ1 containing a latency peptide which blocks its binding to its cognate receptors TGFβ1RI and RII (182,199). PreproTGFβ1 is activated by proteolytic cleavage of the latency peptide (199), a process which can be accomplished by plasmin in the presence of transglutaminase when the preproTGFβ1 is bound to the M6P/IGF-IIR through phosphomannosyl residues on the growth factor (182,195). Following cleavage of the latency peptide, adjacent TGFβ1RI-II can bind the activated growth factor. Impairment of negative growth regulation in altered hepatocytes can result from reduced expression of TGFβ1RI-II and/or of M6P/IGF-IIR. Reduced expression of both types of these receptors characterizes preneoplastic and malignant hepatocytes of HCC in both rats (182,200) and humans (195). In combination, these changes markedly impair the inhibitory action of TGFβ1 on cells of HCC, giving the neoplastic cells a further relative growth advantage as compared to more normal hepatocytes. In addition, some transformed hepatocytes may aberrantly acquire the ability to synthesize active TGFβ1 and to respond to it as a *positive* growth regulator, as has been shown for some other transformed cells (200). HCCs in humans sometimes contain significant levels of both TGFβ1 mRNA and protein (201). As a reflection of increased synthesis by tumor cells, plasma levels of TGFβ1 may be elevated in patients with HCC (202).

Sequential expression of TGFα and IGF-II is directly corroborated in human studies in which expression of both growth factors was examined in the same liver specimens (174). Acquisition of autocrine growth cycles involving TGFα and IGF-II and progressive loss of the growth inhibiting influence of TGFβ characterize altered hepatocytes during the early stages of hepatocarcinogenesis in both rodents and humans. Alterations in these growth factors may provide affected cells with a selective growth advantage, marking those preneoplastic cells in FAH and HN which have the greatest probability to progress to form HCA and HCC. Dysregulation of hepatocyte proliferation may provide a setting in which gene mutations and chromosomal aberrations are facilitated by high rates of cell proliferation.

Increased expression (5- to 20-fold normal levels) of the H-*ras* and *myc* genes have been observed frequently in livers of mice and rats during agent-induced hepatocarcinogenesis (see 203 for early studies). Overexpression of c-*myc* was found in ~10% of FAH (204), and in many HN and HCC of rats exposed to DEN (204–206) compared to unaltered hepatocytes. Although these studies failed to show increased expression of *ras* genes in preneoplastic lesions, the p21 *ras* protein product has been demonstrated immunologically in FAH, HN, HCA and HCC of DEN-exposed rats (207,208). The mechanisms underlying increased expression of proto-oncogenes include amplification of the c-*myc* gene (209,210) and hypomethylation of *ras* genes and *raf* genes in HCCs in rats (211) and B6C3F1 mice (212), respectively. The 5′ flanking region of the H-*ras* gene in hepatocytes of HCC-prone B6C3F1 mice appears to be constitutively hypomethylated (213). Increased expression of the c-*myc* gene in HCC of Beechey ground squirrels chronically infected with GSHV is frequently associated with gene amplification (214), while increased expression of *myc* genes in HCC of WHV-infected woodchucks is related to insertion of WHV genomic sequences into either c-*myc* or N-*myc* genes, or into the N-*myc*2 retroposon (214). IGF-II and *myc* are often overexpressed co-ordinately in HCC of woodchucks (215). Heightened expression of the c-*myc* gene occurs in some cirrhotic nodules in humans (216–218), and elevated c-*myc* expression is a common feature of HCC and of preneoplastic lesions in humans (215–219). The c-*myc* gene may be amplified (219) or hypo-methylated in human HCC (220). Furthermore, susceptibility to HCC in humans segregates with a polymorphism of the L-*myc* gene (221), and amplification of the c-*myc* gene in human HCC is associated with high grade tumors and poor prognosis (219). In contrast to the nearly universal overexpression of c-*myc* in human HCC, the *ras* genes are not often overexpressed (216–218,222,223).

Increased expression of *myc* genes is a common feature of HCC in all species studied. Overexpression of *myc* genes appears to amplify the cellular effects of growth factors. Transgenic mice that overexpress both c-*myc* and TGFα in the liver develop increased numbers of HCC in a shorter time than do comparable mice which express only c-*myc* or only TGFα in the liver (224), and HCC develops at an accelerated rate in woodchucks that coordinately express N-*myc*2 and IGF-II (215). Among other genes that have been found to be aberrantly expressed in human HCC is a gene similar to c-*erb*A (225), as well as, c-*met* (226–228), *neu* (c-*erb*B-2 or HER-2) (229,230), retinoic acid α receptor (231), *CD24* (232) and cyclin D1 (233,234).

In addition to expression abnormalities, *ras* genes are frequently mutated in HCC of mice, less often in rats, and rarely in humans. The H-*ras* proto-oncogene is mutated in 30 to 60% of the spontaneous HCC in B6C3F1 or C3H mice, while mutation of the K-*ras* gene occurs in ~2% of the spontaneous HCC of these HCC-susceptible mice; these mutations are less frequent in

HCC of strains of mice that are more resistant to development of HCC (reviewed in 235). In spontaneous HCC of B6C3F1 mice, mutations predominate in the first base of codon 61; ~60% are GC to TA transversions (CAA to AAA), ~30% are AT to GC transitions (CAA to CGA), and ~12% are AT to TA transversions (CAA to CTA) (235). The frequency of mutations and the pattern of base involvement within codon 61 of the H-*ras* gene vary with the type and dose of carcinogenic chemical (235). HCC in mice exposed to so-called nongenotoxic chemicals (such as peroxisome proliferators) contain few H-*ras* mutations (235). Although mutations in *ras* genes are less common in cells of FAH and HN of mice and increase in frequency in HCA and HCC, these mutations appear to represent a relatively early change during the process of hepatocarcinogenesis in susceptible strains of mice (235). Agent-induced HCCs in the CD-1 strain of mice often contain a larger fraction of mutations in K-*ras* proto-oncogene than do tumors induced by similar agents in B6C3F1 mice, for example (235). Even in the HCC-susceptible mouse strains, *ras* mutations occur in only a fraction (<60%) of HCC (235), indicating that these mutations are not essential for the development of HCC and that other molecular pathways exist. However, these alternative molecular pathways in mice are not known.

In contrast to the situation in mice, mutations of *ras* genes (H, K, and N) are infrequent in HCC of rats and humans. Of interest, the N-*ras* gene is polymorphic in the germ line of Fischer-344 rats, one form of which, N-*ras*3, carries an activating mutation at codon 13 which may confer oncogenic potential (236). N-*ras*3 is not present in the germ line of the relatively more HCC-resistant Wistar strain (237), possibly explaining the somewhat greater susceptibility of Fischer 344 rats to HCC. The K-*ras* gene is mutated in from 5 to 20% of HCC of rats dosed with AFB (236,238,239), 3′-methyl-4-dimethylaminoazobenzene (239), and methyl(acetoxymethyl) nitrosamine (239), but it is uncommonly mutated in HCC associated with other chemical hepatocarcinogens (237, 239–243). H-*ras* and N-*ras* genes are rarely mutated in carcinogen-induced HCCs in rats (237,238,241,244,247). Although one early transfection analysis found that N-*ras* was activated in human HCC (218), this has not been confirmed in other studies (246,247). Mutations in H-*ras* and K-*ras* genes have been identified in only 0–10% of human HCC (247,248). Several other genes of unknown nature (249) or mutated genes involved in known cell growth regulatory pathways (250–253) have occasionally been detected in human HCC, but the import of these mutations is not yet clear. Although *ras* gene mutations in HCC of rats and humans have been less thoroughly examined than in HCC of mice, the apparent species differences in *ras* gene mutation suggest that the molecular pathways that lead to HCC diverge in these species.

The *p53* tumor suppressor gene has been extensively studied in human cancer, including HCC (254). The *p53* gene product functions as a transcription factor and is involved in several areas of cellular function, including the regulation of the DNA synthesis phase of cell cycle transit, DNA repair, and apoptosis (254). G→T transversion mutation in the third base of codon 249 of the *p53* gene ($AGG^{arg} \rightarrow AGT^{ser}$) occurs in a major fraction of the HCCs from human populations of Qidong province in China (255–257) and of Mozambique and Senegal in Africa (257,258), but this specific mutation is less common in other human populations in Southern Africa, Southeast Asia and Japan (257–270) and rare in European and North American populations (259,271–276). Sporadic mutations of the *p53* gene that occur in HCC in Europe and North America involve codons throughout the gene, without the occurrence of hotspots, and G→A transition mutations predominate (254,259). G→A transition mutations affect CpG islands most often and appear to result from spontaneous deaminations (254,259), whereas G→T transversion mutations and microdeletions are usually induced by agent exposure (254,259). The characteristic G→T transversion at codon 249 is highly correlated with dietary exposure to AFB (254–261). HCCs due to HBV or HCV infections alone are not associated with this mutation at *p53* gene codon 249 (265–268,275,277), nor are the HCCs associated with the use of contraceptive steroids (278), exposure to Thoratrast (276), and affliction with the metabolic disease, acute intermittent porphyria (279).

Most of the mutations in the *p53* gene are missense, yielding a protein that retains altered function, and has a prolonged half-life (254,260), apparently associated with a positive cellular growth advantage (254). HCCs that express a mutated *p53* gene are associated with significantly poorer prognosis than are similar tumors that lack these mutations (269). Antibodies to epitopes in wild-type or mutant protein indicate that *p53* protein is present in the cells of many human HCC (273,280–283), consistent with a protein of prolonged half-life, but proteins containing mutations cannot be reliably distinguished from the normal protein by immunohistochemistry of tissues (280,281). G→T transversion mutation of the *p53* gene at the codon 249 hotspot may be an early change in the development of the AFB-associated HCCs in which it is found (254). This opinion is supported by the observation that this mutation is also found in non-neoplastic hepatocytes from livers of patients who live in areas associated with high intake of AFB and high risk of HCC (284). In contrast, random G→A transition mutations in codons other than 249 appear to represent changes that occur late in the progression phase of HCC development in humans (259,262,263,265–269). Supporting this interpretation is the observation that these non-hotspot mutations are more frequent in large, poorly differentiated HCC than in small well-differentiated tumors (265–269). In addition to mutation of one allele, the other allele of the *p53* gene is often deleted in human HCC (256–258,262,264–269,271,285–293); deletion of the normal *p53* allele appears to take place after the mutation of the other allele, occurring later in the process of HCC development (256,269,285–290). It is curious that although the Li-Fraumeni Syndrome is often associated with germ-line mutation or deletion of one allele of the *p53* gene and with increased risk of developing neoplasms of several tissues, affected patients are not at increased risk to the development of HCC (294).

Although overexpression of *p53* protein often has been found in hepatoblastomas of children (280), demonstration of gene mutation has been variable. In one series no mutations were found in exons 5 to 9 among 15 tumors, although *p53* protein was overexpressed (280). However, in other studies a G→T transversion at codon 249 was detected in one of three tumors (274) and a deletion of codon 72 was detected in one of five informative tumors (272), but no mutations were found (272). In contrast, a recent study detected mutations in exons 5 to 8 of the *p53* gene in 9 of 10 hepatoblastomas occurring in Japanese children, with eight identical mutations occurring in a hotspot at codon 157 (295). In addition, two missense mutations in codon 244 and one each in codons 273 and 279 were found (three tumors contained double mutations) (295). Eleven of the 12 mutations were G→T transversions, and the *p53* protein was over-expressed in nine (295). These observations suggest that mutation of the *p53* gene may also have an important role in the development of hepatoblastoma in humans.

Several other tumor suppressor genes may also influence hepatocarcinogenesis in humans. Mutation (296) and/or LOH (285,293,296–298) of an Rb-1 allele occurs in some HCC of humans, but HCC has not been identified as a second neoplasm in survivors of retinoblastoma (299). HCC and hepatoblastoma occur as second neoplasms in patients with Wilms' tumor (300,301), and one or more WT-associated loci may be deleted in these liver tumors (297,302–306). Hepatoblastoma is also a common neoplasm in first-degree family members of patients with familial adenomatous polyposis (307), and the APC gene is often somatically mutated and/or shows LOH in sporadic hepatoblastoma (308). Marker loci near the APC locus are also deleted in some HCC (256,285,289,290). The M6P/IGF-IIR gene is often mutated in HCC (197), and the remaining allele may be deleted (196,197), suggesting that this gene also has a tumor suppressor function in HCC development. Mutation and allelic deletion of the M6P/IGF-IIR gene is an early change, occurring in HCA as well as in small HCC (196).

Indications that additional unidentified tumor suppressor genes are involved in the pathogenesis of HCC in humans include LOH and non-random chromosomal structural abnormalities at sites of unknown genes that recur repeatedly in HCC of humans. The chromosomes and chromosomal regions that have been found to be abnormally structured or deleted in HCC in humans (309–328) are listed in Table VII.

Complete karyotypic analyses of individual chromosomes in human HCC are uncommon. Complex structural aberrations involving chromosomes 1, 5, 6, 9, 13, 16 and 22 were found in one HCC (319) (Table VII). Breaks and deletions were often located in chromosome 1p22 and 1p32 (309,319,321). Structural aberrations involving chromosome 17 have been identified several times, including translocations between chromosomes 17 and 18 (322), 17 and *X* (323), and 17 and 7 (324). Consistent with these observations, chromosome 17 was found to be numerically abnormal by use of a chromosome-specific probe in 44% of HCCs (325). Genomic scanning studies indicate even more extensive qualitative and quantitative aberration of genomic content (326), although the specific chromosomes were not identified. Both structural and numerical (327,328) chromosome aberrations have been identified in hepatoblastomas, involving chromosomes 2, 9, 11, 17 and 20. Many LOH and chromosome aberrations are coincident with sites of HBV integration (267,268,290,297,303,310,311,319,323,324), which produces small deletions in the human genome (329). Similarly located LOH also occur in HCC related to HCV infection (267,286,311) in which genomic integration does not occur.

Accumulation of chromosomal structural abnormalities and deletions are associated with the development of malignancy and the further progression of HCC in humans. When appropriately examined, LOH have not been found in HCA at most of the same loci on the chromosomes listed in Table VII (330), the exception being the M6P/IGF-IIR locus which is homozygous or hemizygous in some HCA (196). In contrast, a 17p LOH occurred in an HCC while a synchronously occurring HCA lacked this same LOH (331). Accumulation of LOH at multiple alleles is associated with the progression from small, well-differentiated HCC to large, poorly differentiated HCC. Poorly differentiated HCC may contain LOH involving up to 8 alleles, with each poorly differentiated tumor containing LOH in an average of 3 alleles

Table VII. Chromosome abnormalities in human HCC and hepatoblastoma

Chromosome	Numerical aberrations (309,319, 321,328)	Structural aberrations, deletions, translocations (309,319–324,327)	Loss of heterozygosity (195–197,247,249,262, 267,285–293,296–298, 302–306,309–319,328,331)	Possible or actual genes involved
1		tl:? (p32:?) del 1(p22) del 1 (p32–p36)	1p, 1p35–36 1q 42–43	L-myc (1p32)
4	−		4p 4q, 4q32, 4q11–23	AFP/ALB/αFGF
5	−	t5;? (q34;?) t5;9(–;–)	5p 5q, 5q35–qter	APC (5q21)/MCC
6	−	del 6(q13–qter)	6q26–q27	M6P/IGF-IIR (6q26)
7	+	tl7;7 (p13;p14)		
8	−	inv 8(q10)	8q	c-*myc* (8q24)
9	−	t5;9 inv 9(p12:q12)		
10	−		10q	
11	−,+	dup11p15 del 11(p13–p14) del 11p11 t 11:22	11p, 11p13–p15.1	WT-1(11p15), IGF-II, others
12	−			
13	−,+	der13;15 (q10;p10)	13q, 13q12, 13q12–q31	*Rb*-1 (13q14)
15	−,+	der13;15 (q10;p10)		
16	−	del 16q der 16q24	16p 16q22–24	E-cadherin (16q22.1)
17	−	t17;7 (p13;p14) t17;18 (q25;q11) t17;X del 17(p12)	17p, 17p13, 17p13–pter	*p53* (17p13)
18	−,+	t17;18 (q25;q11)		
19				
20	+			
21	−,+			
22		t22;? (q12;?)	22q, 22q11–12	
X	−	t17:X		

(286). Nearly all large, poorly differentiated HCC contain LOH at alleles on chromosomes 4q, 13q, 16q, and 17p (285–289). Clearly not every HCC shows the same pattern of genetic aberrations, suggesting that a combination of several genetic abnormalities must accumulate to dysregulate hepatocyte proliferation and differentiation; no single pattern of genetic abnormality yet appears to predominate.

Mutations and locus deletions of the *p53* gene appear to have a less pervasive role in the development of HCC in rodents than in humans; *p53* mutations analogous to the hotspot mutations at codon 249 in humans are not found in HCC of woodchucks and Beechey ground squirrels infected with species-specific hepadnaviruses related to HBV (332), in HN (333) or HCC of rats (334), and in HCC of non-human primates (335) exposed to AFB, suggesting that this dimension of molecular pathogenesis of HCC may be specific to humans. However, transition mutations in other *p53* codons were found in some HCC of AFB-exposed rats (238,334), in an HCC of one Beechey ground squirrel (332), in an HCC of one nonhuman primate exposed to AFB (335), and in HCCs of several primates exposed to 2-amino-3-methylimidazo(4,5-f)quinoline (336). Other studies on mutation of the *p53* gene in HCC of rats have yielded highly variable results. Accumulation of 'mutant' *p53* protein was found in FAH induced by exposure of rats to DEN (337) and in oval cells stimulated by 2-acetylaminofluorene (AAF)-exposure (338). Subsequent studies found frequent mutations in the *p53* gene in HCCs induced in rats by AAF or N-nitroso-AAF (339), or by feeding a choline-devoid diet (340). Hotspot mutations were found in *p53* codons 234 and 294 of nearly all HCC induced in rats by tamoxifen (141). In sharp contrast, other studies have found no *p53* mutations in HCC of rats induced by various other carcinogens (238,341,342). Whether these mutations are agent-specific, as for the AFB-related codon 249 mutation in humans, requires further study. Mutations in the *p53* gene have apparently never been identified in murine HCC (343–345), including hepatoblastoma, which in mice is a highly progressed and poorly differentiated variant of HCC (4,14,346). Furthermore, germ-line deletion of one or both alleles of the *p53* gene in mice or deletion of one allele combined with mutation of the remaining allele does not increase susceptibility to the development of spontaneous (347) or chemically induced (348) HCC. Abnormalities in the Rb gene occur infrequently in HCC of rats (349), and germ-line deletions or mutations of the Rb gene in mice are not associated with increased susceptibility to HCC (350). Furthermore, HCC susceptibility is not increased in mice bearing germ-line mutations in both Rb and *p53* genes (351).

Locus deletions have been less intensively examined in HCCs of rodents than in human HCCs. A study of spontaneous and chemically induced HCC in B6C3F1 mice detected LOH of microsatellite and other markers on chromosomes 2, 5, 6, 8, 9, 14 and 18 (Table V) in about one-third of the tumors; however, the most frequently affected locus was deleted in only 9% of tumors (352). Two other studies using similar techniques have identified only one LOH among 102 HCCs and 27 HCAs produced in mice of strains MSM and C3H, or in F1 crosses, including B6C3F1 (353) and C3H/He×*Mus spretus* (354). In contrast, LOH on chromosomes 1, 5, 7, 8, 12, and 13 were found in 100% of 30 HCC induced in a cross between a transgenic line derived from *M castaneus* or C57BL/6 containing the SV40 early T-antigen transgene and *Musus spretus*, in which 575 mapped loci could be identified (355,356) (Table V). Hepatocytes are often aneuploid in HCC arising in doubly transgenic c-*myc*/TGFα mice, and many cells contain breaks and translocations involving chromosomes 1, 4, 7, 12 and 19 (357) (Table V). Locus deletions in genomic DNA of HCCs produced in rats have apparently not been investigated with the use of microsatellite markers. However, chromosomes of rat HCC frequently contain non-random structural abnormalities by karyotypic analysis (358–360). Rat chromosomes 1, 2, 3, 4, 6, 7, 8, 10 and 11 contain nonrandom structural abnormalities in a fraction of HCC (Table V).

Taken together, these observations on aberrations in known and putative tumor suppressor genes during hepatocarcinogenesis in humans and rodents suggest major differences in the molecular pathogenesis of HCC in these species. However, chromosomal abnormalities (deletions, breaks, translocations) in humans, rats and mice involve several regions containing gene clusters that are syntenically conserved among all three species, suggesting the possibility that common tumor suppressor genes may be involved in the pathogenesis of HCC in both rodents and humans (361). Evidence supporting this possibility arises from studies in which the tumorigenicity of rat HCC cells that contain structurally abnormal chromosome 1 (which contains

extensive sequences of syntenically conserved genes in common with human chromosome 11 (361)) is reversed by inserting an intact human chromosome 11 into tumor cells by microcell fusion (362). Recent studies suggest that the common tumor suppressor gene is the WT-1 locus, together with a second locus on human chromosome 11 that regulates the expression of the WT-1 gene (363).

Natural history

The natural history of HCC begins with the occurrence of its earliest antecedent lesions and continues through the initial appearance of malignant cells until the affected individual dies from the effects of the neoplasm. Delineation of the earliest preneoplastic lesions that precede the development of HCC is less detailed in humans than in rodents.

HCC usually occurs in humans who have suffered from chronic hepatitis; the end result of which is cirrhosis. Although not a required precursor of HCC in humans, cirrhosis of any morphological type or etiology is associated with increased risk of HCC (40,41); however, the *magnitude* of risk reflects the specific etiology of the cirrhosis (40), suggesting that the nature of the causative agent is determinant. HCC develops at the annual rate of 5 to 8% of the population with cirrhosis due to HCV infection (73,364–367), and a similar level of risk is associated with cirrhosis due to hemochromatosis (40,98). Cirrhosis due to chronic HBV infection is associated with a slightly lesser risk to HCC (40,73,364–368), and cirrhosis related to other causes such as chronic alcoholism, chronic autoimmune hepatitis or Wilson's disease carries much smaller risks (40,80,100,101). HCC occurs in the setting of chronic hepatitis without cirrhosis, but accompanied by foci to dysplastic hepatocytes and by HN (38,46,369), in Alaskan natives who are often infected with HBV at young ages (370). This observation suggests that the events leading to HCC may develop in humans without the production of recurrent hepatocellular necrosis of an extent sufficient to lead to cirrhosis.

HCCs that occur in either cirrhotic or noncirrhotic livers are closely similar (371,372). Nevertheless, the natural history of HCC differs somewhat in human populations that are distinguished by either a low or a high incidence of this tumor. In the geographic areas in which exposure to AFB is significant and/or chronic infections with HBV and HCV virus are highly prevalent, HCC occurs in the absence of cirrhosis somewhat more frequently and at an earlier age than in areas in which exposure to these etiologic agents is less intense (3,38). HCCs begin to appear early in life in high-incidence areas and reach a peak incidence in the fourth or fifth decades (3), often in the absence of cirrhosis. HCC is a disease of old age in low-incidence areas, with the peak incidence occurring in the fifth to seventh decades of life (3), and it is almost always associated with cirrhosis. In both high- and low-incidence areas, HCC predominates in males, with male-to-female ratios of >3–4 to 1 (3). Part of the diffference in age-dependent incidence may reflect the specific etiology; in Japan peak incidence of HCC occurs at a younger age in HBV-infected patients (52 ± 13 years; M ± SD, $n = 23$) than in HCV-infected patients (62 ± 7 years; $n = 145$) (367).

Age at the time of infection with HBV or HCV is an important determinant of the age at which HCC occurs. For example, Alaskan natives chronically infected with HBV typically acquire infection perinatally (370), and many develop HCC at an early age, with an elevated incidence detectable in the first decade of life (369). Acquisition of chronic HBV infection later in life, as in Japan (366), is associated with the later development of HCC. The temporal relationship between chronic infection with HCV and the development of HCC, as well as the variability in the clinical progression along this sequence, is illustrated by a study of Japanese patients receiving blood transfusions and subsequently shown to be infected with HCV (373). All but two of the 21 patients studied were older than 25 years when they received blood transfusion. The time from blood transfusion to detection of chronic hepatitis was 10.0 ± 11.3 years (M ± SD; $n = 45$; range 1 to 46 years), to detection of cirrhosis was 21.2 ± 9.6 years ($n = 23$; range 8 to 53 years), and to detection of HCC was 29.0 ± 13.2 years ($n = 21$; range 13 to 62 years). Three of 21 HCCs (14%) developed in the absence of cirrhosis, but each of these patients had chronic hepatitis (373).

After becoming clinically apparent, HCC is a rapidly progressive and uniformly fatal disease in humans, typically associated with severe symptoms, numerous paraneoplastic complications and metastasis (6–8,364–368). Survival time of patients varies with the size of the tumor at diagnosis and with its growth rate. The growth rates of HCCs in humans vary markedly, with tumor volume-doubling times that range from 1 to 19 months (366). Survival of patients with both HCC *and* cirrhosis depends not only on the growth and metastasis of the tumor, but also on the severity of the cirrhosis (364–368), since both cirrhosis and HCC are potentially lethal diseases. HCCs usually reach larger sizes in noncirrhotic livers than in cirrhotic livers (6,7,371,372), often forming large, single lesions in noncirrhotic livers. In the absence of cirrhosis, HCCs may be clinically silent until they reach a massive size; in contrast, cirrhosis often produces symptoms that cause patients to seek medical care before HCCs develop or are clinically apparent.

The natural histories of HCC and HCA differ strikingly in humans. HCA is uncommon in humans, and rarely occurs in synchrony with HCC; HCA is not recognized as a complication of cirrhosis in humans, as is HCC. HCA occurs most frequently in human females of childbearing age (375), and in this population the incidence of HCA is augmented slightly by the use of oral contraceptives containing mixtures of estrogenic and progestational steroids (376,377). In human males, HCA is rare, occurring most often in association with the use of androgenic anabolic steroids (378,379). Development of HCC in HCA is an uncommon, but recognized, occurrence in humans (7,8,380). Not only have pathologic examinations of HCA occasionally revealed HCC (7,8,379), but epidemiological studies show that chronic use of contraceptive steroids that are risk factors for HCA also carry an increased risk for HCC (376,380).

HCC in rodents generally appears to be less rapidly progressive and life-threatening even after detection, and much more common than is HCC in humans. For example, in mice, HCC has a relatively benign course, progressing so indolently that the expected life span of an affected animal is not usually shortened, and metastases occur late in the course of the disease (131,132). The natural histories of HCA and HCC in mice and rats are nearly identical, unlike the situation in humans; in rodents both tumors occur concurrently and the incidences of each increase with age (25,26,131,132,381,382). Even in the absence of known exposure to carcinogens, up to 45% of male B6C3F1 mice are affected by HCA and/or HCC at 80 to 108 weeks of age, while <10% of females of this age bear these hepatocellular tumors, and in mice of both sexes 30 to 50% of all spontaneous hepatocellular tumors are HCAs (131,132). As many as 10% of male Fischer-344 rats and a slightly smaller fraction of females harbor HCA and HCC at 102 to 107 weeks of age even in the absence of carcinogen exposure, and 80 to 90% of the spontaneous hepatocellular tumors in rats of both sexes are HCAs (381,382). The incidence of HCA and HCC spontaneously occurring (that is, without known exposure to carcinogens) in either mice or rats of the strains commonly used in carcinogenicity tests is much higher than is the incidence of these tumors occurring in any carcinogen-exposed human population that has been studied (3).

The natural histories of chemically induced HCA and HCC in rodents are similar in their major features to comparable spontaneously developing tumors. Notably, HCA and HCC often occur concurrently (synchronously) in rodents exposed to carcinogenic agents (25,26). As with humans, HCC predominates in male mice and rats by ratios of >3–4 to 1 (male to female), but unlike humans, HCA also occurs most frequently in male (rather than female) mice and rats. The sex ratios and the relative frequencies of benign to malignant tumors are similar in mice and rats for both spontaneous and treatment-induced hepatocellular tumors. Cirrhosis is rare in mice and uncommon in rats on carcinogenic regimens, and metastasis from HCC in rodents is infrequent (25,26). Non-neoplastic proliferative lesions, including FAH and HN, also coincide temporally with both the spontaneous and agent-induced occurrence of HCA and HCC in both rodent species (25,26,131,132,381,382). When carcinogen exposures are started in juvenile test animals, as in the typical bioassay protocol, chemically induced HCCs occur late in the life-span of mice and rats (25,26,158); prenatal or perinatal exposures of mice to carcinogenic chemicals results in somewhat earlier occurrence of HCC (383,384). In rats, FAH develop at 1 to 4 months, HN at 3 to 10 months, and HCC at 12 to 20 months after beginning various hepatocarcinogenic regimes (158).

The natural history of hepatoblastoma differs strikingly in humans and rodents. In humans hepatoblastoma, which originates during liver embryogenesis and may be present at birth, occurs almost exclusively in children under the age of 3 years (7,8), but this tumor occurs in adult rodents in conjunction with, and apparently as a poorly differentiated variant of, HCC (5,14,346).

HCCs in rodents and humans share a slow development, passing through multiple steps that require most of the host's lifespan unless tumor formation began during fetal development or early postnatal life. Despite this common general pattern, the natural history of HCC and of related preneoplastic and neoplastic lesions in humans differs considerably from that in mice and rats, reflecting substantial differences in cellular and molecular pathogenesis among these species.

Discussion

As outlined in this review, hepatocarcinogenesis is a heterogeneous molecular and cellular process that appears to involve the accumulation of aberrations in several genes and the clonal proliferation of various populations of altered cells before a

malignant neoplasm develops. Heterogeneity in the development of HCC is made even more complex since different combinations of aberrantly expressed genes may dysregulate cellular growth, and neoplastic cells may originate from either stem-like cells or differentiated hepatocytes. Some features of hepatocarcinogenesis are similar in mice, rats and humans, but several steps in the process diverge among these species. The HCCs that ultimately develop are histologically similar in all three species, but the evolving cellular lesions that precede HCC differ among rodents and humans. The common tissue settings for the development of HCC in humans, in which chronic hepatitis and cirrhosis are nearly universal features, clearly differ from the setting for HCC in rodents. The relationship of HCA to HCC differs markedly in rodents and humans, and hepatoblastomas represent distinctly different neoplasms in these species. However, the aberrant populations of non-neoplastic hepatocytes that precede and accompany the development of HCC in both humans and rodents, seem to share phenotypic properties, suggesting that the early cellular lesions are similar among rodents and humans.

Even among individual animals, either rodents or humans, hepatocarcinogenesis shows molecular heterogeneity. The combinations of abnormal genes that are effective, and the order in which they become aberrant, may vary from individual to individual, as in colon cancer (385). The earliest molecular alterations that appear to be associated with the subsequent development of HCC in both rats and humans include aberrant regulation of the c-*myc* proto-oncogene and of the growth factors TGFα, IGF-II and TGFβ. Loss of expression of the M6P/IGFII-R gene also is a frequent early change in preneoplastic hepatocytes in rodents and humans. Although aberrant expression of these genes is frequently observed in each species, such changes are not found in all instances of HCC. Additional genetic aberrations during the progression stage of hepatocarcinogenesis appear to involve different regulatory pathways in rodents and humans. The molecular pathogenesis of HCC in mice is characterized by frequent mutations in proto-oncogenes, particularly the *ras* group, in contrast to the process in rats and humans in which *ras* mutations are uncommon. The molecular development of HCC in humans is associated with extensive alterations in known and putative tumor suppressor genes, including the *p53* gene, in contrast to the situation in rodents. Failure to detect LOH of known and putative tumor suppressor loci in HCC of rodents may result from the predominant use of highly inbred strains of mice and rats in which both alleles of each gene are identical. Studies using interstrain F1 hybrids of genetically divergent strains of mice suggest that LOH of specific genes also occurs in murine HCC. The identification of HCC susceptibility loci that act by controlling the rate of clonal proliferation of preneoplastic cellular lesions seems to be unique to mice. Similar susceptibility loci have not been identified in rats or humans.

In addition to apparent variations in molecular pathogenesis of HCC, mice and rats also differ from humans in several other ways not detailed in this review that may impair their use as precise models of humans for the testing of hepatocarcinogenic chemicals. Although it is well known that certain strains of mice are more susceptible to HCC than are other strains, mice and rats of all strains appear to be generally more susceptible to development of cancer in all tissues than are humans. For example, 40% to 50% of mice and rats which develop cancer express neoplasms in more than one organ or tissue simultaneously (1), a situation that occurs in $<5\%$ of humans who have cancers at various sites (386,387), including the liver (388). In humans, simultaneous occurrence of neoplasms in multiple tissues in an individual is a criterion used to identify families in which individual members suffer from genetic conditions conferring increased susceptibility to cancer (389). In accord with this suggestion HCC develops spontaneously in over 10% of Fischer 344 male rats and 40% of B6C3F1 male mice during their lifespan, whereas $<10\%$ of human males develop HCC even in populations that suffer from the highest agent-induced incidence of this disease. Possibly the species-dependent differences in the frequency of HCC development and in the multiplicity of tissues affected by cancer are also related to the striking differences in the time required for cancer development in rodents and humans. HCC usually develops late in the lifespan of all three species, which means that this tumor evolves much more rapidly in mice and rats than in humans. The incidence of HCC in humans bears a logarithmic relationship to host age, a result suggesting that five to seven discrete steps evolve in a time-dependent manner during hepatocarcinogenesis in humans (390). The multiple cellular and molecular events that occur during hepatocarcinogenesis in both rodents and humans may provide the discrete biological steps that are responsible for this mathematical relationship. More rapid development of HCC in rodents than in humans suggests that fewer steps are necessary and/or that the transition between individual steps is more rapid in rodents. The answer to this conundrum is not yet available. However, a broad range of evidence indicates that rodent cells are genomically more unstable and more readily immortalized and neoplastically transformed than are human cells (391). A general mechanism for genomic instability and transition to 'immortality' may involve the replication of telomeric ends of chromosomes by the enzyme telomerase. Replication of DNA during the cell cycle efficiently reproduces all of the cell's genomic DNA except for the ends of chromosomes, the telomeres. Telomeres, composed of repeated TTAGGG sequences, are replicated by the enzyme telomerase, which is not expressed in somatic cells of adult humans (392). As a consequence, the telomeres of human somatic cells appear to shorten by a few bases with each replicative cell cycle, and they may be lost after a sufficient number of cycles (392). Chromosome ends lacking telomeric DNA are analogous to a double-strand break elsewhere in a chromosome, and provide the basis for structural rearrangements (392). Cells that lack telomeres enter a stage of 'crisis,' during which most die but a few may acquire an aneuploid state, which when coupled with the re-expression of telomerase may enable them to become 'immortal' (392). Although telomerase is not detectable in normal liver tissue of humans, it is expressed at low levels in chronic hepatitis and at higher levels in HCCs (393), sufficient to maintain short telomeres in rapidly cycling malignant cells (394). In contrast, normal hepatocytes of adult rodents express high levels of telomerase activity (395,396), indicating that telomerase activity is less stringently regulated in somatic cells of rodents than of humans. This species-related difference in regulation of telomerase activity may play a role in making rodents more susceptible to cancer than are humans.

The imprinting of some genes that appear to be critically involved in the pathogenesis of HCC also differs in cells of rodents and humans. Expression of the two parental alleles of an imprinted gene is differentially regulated by an epigenetic

process, such as methylation (397). Imprinting, which renders a gene functionally monoallelic, makes an affected cell more susceptible to the inactivation of the expressed allele by mutation or deletion from a 'single hit'. At least two genes importantly involved in development of HCC in both rodents and humans, IGF-II and M6P/IGF-IIR, are differently imprinted in hepatocytes of these species. In rodents, both genes are imprinted in hepatocytes during both embryogenesis and adulthood (397–399). In contrast, although both genes are imprinted during embryogenesis of the liver in humans, they are biallelically expressed in hepatocytes of adult humans (192,195). Loss of imprinting (LOI) of IGF-II during late fetal life may lead to the biallelic overexpression of this growth factor and cause dysregulated cellular growth of hepatocytes; such an event is common in Wilms' tumors (397), but occurs only occasionally in hepatoblastoma in humans (192–194). Elevated levels of IGF-II in hepatoblastoma and HCC in humans more often results from altered IGF-II promoter usage (192–194) or from inactivation of the M6P/IGF-IIR gene (182,195–197). Mutation and/or deletion of the M6P/IGF-IIR gene appears to be an early event in the pathogenesis of HCC in both rodents and humans (182,195–197). Imprinting of the M6P/IGF-IIR gene in hepatocytes of rodents makes them more susceptible to the loss of the remaining allele than are humans.

The majority of carcinogenic chemicals require metabolic activation to chemical species that covalently bind to DNA (400). Species-dependent variations in response to chemical hepatocarcinogens reflect extensive differences among rodents and humans in the absorption, distribution, metabolism, and excretion of chemicals (401). Although metabolic activation of chemicals occurs through multiple mechanisms, oxidation reactions involving cytochrome P-450 enzymes play a major role (401). The livers of all animals contain a rich repertory of cytochrome P-450 enzymes (400). Metabolism of many chemicals by specific cytochrome P-450 enzymes varies considerably among the three species considered here (402–405). Variations in enzymes that secondarily metabolize products resulting from the action of P-450 enzymes also importantly influence carcinogenicity of chemical metabolites (111–114). The combined differences among species in the actions of phase I and phase II enzymes sometimes lead to the production of different amounts and types of metabolites in hepatocytes of rodents and humans, and result in species-dependent differences in mutagenicity and carcinogenicity (111–114,405). Additionally, the dose of chemicals to which rodents are exposed in laboratory tests and to which humans are exposed environmentally differ greatly; rodents are exposed to high doses in experimental studies, whereas humans are typically exposed to low doses in the environment or workplace. The high chemical doses to which rodents are exposed may distort the usual patterns of chemical metabolism, as well as the process of carcinogenesis.

Whatever the basis, major differences characterize the known causes of HCC in humans and rodents. The main risk factors for HCC in humans are chronic infections with the hepatotropic viruses HBV and HCV, while chemicals comprise the major hepatocarcinogens of mice and rats. As a result of environmental contamination, workplace exposure, medical therapy, and personal habits, humans are exposed to many of the chemicals that are potent hepatocarcinogens for rodents. Although the database that includes carcinogenicity assessments of the same chemicals in all three species is small, the available results indicate a remarkable lack of concordance in the occurrence of chemically induced HCC in humans and rodents. It is obvious that the human liver is not immune to the carcinogenic actions of chemicals, since several chemicals that are potent hepatocarcinogens for humans are well recognized. Nevertheless, the poor level of interspecies concordance in chemically induced hepatocarcinogenesis suggests that rodent tests may not accurately identify the particular chemicals that pose a HCC risk to humans. Rodent bioassays may detect some chemicals that *do not* pose a human risk of HCC (false-positives), and, as well, they also may *not* detect some chemicals that do pose a human risk of HCC (false-negatives). Species-dependent variations in chemical metabolism, including dosage differences, amplified by differences among species in the pathogenesis of HCC, provide the biological basis for making rodents poor surrogates for detecting chemicals that are potential human hepatocarcinogens.

Although currently available evidence does not demonstrate that chemicals at levels that pollute the environment have a significant role in etiology of HCC in humans, the possibility of an effect of environmental chemicals on the total risk of HCC for humans cannot be confidently excluded. The etiology of HCC in humans is clearly multifactorial. Multifactorial causation is mechanistically complex, and our understanding is poor (406). Two or more chemicals may interact in multiple ways, either to augment or reduce risk of cancer. Even the minor components of some multifactorial mixtures may exert a determinative role in the overall hepatocarcinogenicity of a complex mixture of carcinogenic agents. Furthermore, biological agents, such as HBV, may interact with chemicals through effects on various cellular and molecular steps involving the metabolic activation of chemicals (407–409) and on other features of hepatocytic metabolism involved in the pathogenesis of hepatocarcinogenesis (410–412).

Interspecies concordance in the organ/tissue sites of cancer such as the liver, has been suggested to be unimportant in the extrapolation of risk from rodents to humans (413). The organism is obviously the ultimate object of risk identification, since cancer originating in any organ or tissue, not just the liver, is potentially lethal. However, unlike rodents, cancers in humans typically involve single sites only, making site-specific cancers (such as HCC) the objects of concern for the identification of specific carcinogens in assays employing rodents and for the extrapolation of inferred risk to humans. Interspecies concordance is poor even when cancers occurring at any site are included in the assessment, and concordance of the liver site is less good among rodents and humans, even though the liver is a major site of cancer in each species. Further interspecies comparisons of several of the major sites of primary cancer in humans and rodents could provide data to assess whether the ability to extrapolate risk from rodents to humans is any better for the other major organ sites in humans than it is for the liver. Preliminary evidence suggests that the pathogenesis of the development of site-specific cancers in other tissues may diverge as much among mice, rats and humans as it does for the liver. Recent studies suggest that colon cancer develops by molecular pathways that differ in mice and humans. Analysis of genetic aberrations in chemically induced colon cancer in susceptible mice shows that none of the six or seven genes commonly mutated or deleted in some combination in colon cancer in humans (385) are affected in these strains of mice (414). Other studies suggest that the molecular pathogenesis of intestinal cancer

(not specifically colon cancer) has both common and divergent features in mice and humans. For example, mutation (415) or deletion (416) of the APC gene, an early cellular step in the development of colon cancer in humans (385), increases the susceptibility of mice to the development of intestinal adenomas. Furthermore, mice bearing both a mutation in the APC gene (MIN mice) and a deleted mismatch repair gene (Msh2) show accelerated development of intestinal tumors (417). Nevertheless, the natural history of these intestinal tumors in MIN mice, in which the Msh2 gene is also mutated, differs markedly from that of colon cancers in humans; the majority of the adenomas occur in the small bowel in mice, rather than in the colon, as in humans, and they infrequently become malignant (415–417). Additionally, germ-line mutation and/or deletion of the *p53* gene, a molecular event also frequently involved in the pathogenesis of colon cancer in humans (385), does not effect the development of intestinal cancer in mice (347,414). Mice, rats, and humans also seem to differ in terms of hormonal regulation of mammary carcinogenesis (418), suggesting that molecular pathways of mammary carcinogenesis may also diverge among these three species. Further comparative studies of specific-site neoplasms in all three species, including more detailed and comprehensive analysis that integrates knowledge of causes, cellular and molecular lesions, and natural history, will be necessary to determine how far off the mark are rodents as reliable indicators of human risk at specific sites.

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The number of publications relating to aspects of this review are enormous. In order to limit the length of the reference list I have cited other reviews when available. On some subjects that have been discussed extensively I have been selective in the citation of literature. I apologize to the many authors whose original work is not cited.

References

1. Gold,L.S., Slone,T.B., Manley,N.B. and Bernstein, L. (1991) Target organs in chronic bioassays of 533 chemical carcinogens. *Environ. Health Perspect*, **93**, 233–246.
2. Huff,J., Cirvello,J., Haseman, . and Bucher,J. (1991) Chemicals associated with site-specific neoplasin in 1394 long-term carcinogenesis experiments in laboratory rodents. *Environ. Health Perspect*., **93**, 247–270.
3. Parkin, D.M., Pisani,P. and Ferlay,J. (1993) Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int. J. Cancer.*, **54**, 594–606.
4. Frith,C.H. and Ward,J.M. (1980) A morphologic classification of proliferative and neoplastic hepatic lesions in mice. *J. Environ. Pathol. Toxicol.*, **3**, 329–351.
5. Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences. Histologic typing of liver tumors in the rat. *J. Natl Cancer Inst.*, **64**, 178–206.
6. Edmundson,H.A. and Steiner,P.E. (1954) Primary carcinoma of the liver. A study of 100 cases among 48,900 necropsies. *Cancer*, **7**, 462–503.
7. Craig,J.R., Peters,R.L. and Edmondson,H.A. (1988) *Tumors of the liver and intrahepatic bile Ducts*, Armed Forces Institute of Pathology, Washington, DC, pp. 280.
8. Altmann,H.W. (1994) Hepatic neoformations. *Pathol. Res. Pract*., **190**, 513–577.
9. Goldfarb,S. and Pugh,T.D. (1986) Multistage rodent hepatocarcinogenesis. *Prog. Liver Dis.*, **8**, 597–620.
10. Danielsen,H.E., Steen,H.B., Lindmo,T. and Reith,A. (1988) Ploidy distribution in experimental liver carcinogenesis in mice. *Carcinogenesis*, **9**, 59–63.
11. Schwarze,P.E., Saeter,G., Armstrong,D., Cameron,R.G., Laconi,E., Sarma,D.S.R., Preat,V. and Seglen,P.O. (1991) Diploid growth pattern of hepatocellular tumors produced by various carcinogenic treatments. *Carcinogenesis*, **12**, 325–327.
12. Anti,M., Marra,G., Rapaccini,G.L. *et al*. (1994) DNA ploidy pattern in human chronic liver diseases and hepatic nodular lesions: flow cytometric analysis on echo-guided needle liver biopsy. *Cancer*, **73**, 281–288.
13. Cottier,M., Jouffre,C., Maubon,I., Sabido,O., Barthelemy,C., Cuilleron,M., Veyret,C., Laurent,J.-L. and Audigier,J.-C. (1994) Prospective flow cytometric DNA analysis of hepatocellular carcinoma specimens collected by ultrasound fine needle aspirations. *Cancer*, **74**, 599–605.
14. Diwan,B.A., Ward,J.M. and Rice,J.M. (1992) Origin and pathology of hepatoblastoma in mice. In Sirica,A.E. (ed.), *The role of cell types in hepatocarcinogenesis*, CRC Press, Boca Raton, pp. 71–87.
15. Howell,S., Wareham,K.A. and Williams,E.D. (1985) Clonal origin of mouse liver tumors. *Am. J. Pathol*., **121**, 426–432.
16. Lee,G.-H., Nomura,K., Kanda,H., Kusakabe,M., Yoshiki,A., Sakakura,T. and Kitigawa,T. (1991) Strain specific sensitivity to diethylnitrosamine-induced carcinogenesis is maintained in hepatocytes of C3H/HeN[←→]C57BL/6N chimeric mice. *Cancer Res*., **51**, 3257–3260.
17. Tsunashima,K., Endo,Y., Asakura,H., Kanda,H., Nomura,K., Kitagawa,T. and Kominami,R. (1996) A novel clonality assay for the mouse: application to hepatocellular carcinoma induced with diethylnitrosamine. *Mol. Carcinogenesis*, **15**, 33–37.
18. Shafritz,D.A., Shouval,D., Sherman,H.I., Hadziyannis,S.J. and Kew,M.C. (1981) Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies on percutaneous liver biopsies and post-mortem specimens. *N. Engl. J. Med.*, **305**, 1067–1073.
19. Esumi,M., Aritaka,T., Arii,M., Suzuki,K., Tanikawa,K., Mizuo,H., Mima,T. and Shikata,T. (1986) Clonal origin of human hepatoma determined by integration of hepatitis B virus DNA. *Cancer Res.*, **46**, 5767–5771.
20. Tsuda,H., Hirohashi,S., Shimosato,Y., Terada,M. and Hasegawa,H. (1988) Clonal origin of atypical adenomatous hyperplasia of the liver and clonal identity with hepatocellular carcinoma. *Gastroenterol*., **95**, 1664–1666.
21. Aoki,N. and Robinson,W.N. (1989) State of the hepatitis B viral genomes in cirrhosis and hepatocellular carcinoma nodules. *Mol. Biol. Med*., **6**, 395–408.
22. Kawai,S., Imazeki,F., Yokosuka,O., Ohto,M., Shima,S., Kato,N. and Omata,M. (1995) Clonality in hepatocellular carcinoma: analysis of methylation pattern of pleomorphic X-chromosome-linked phosphoglycerate kinase gene in females. *Hepatol*., **22**, 112–117.
23. Gaffey,M.J., Iezzoni,J.C. and Weiss,L.M. (1996) Clonal analysis of focal nodular hyperplasia of the liver. *Am. J. Pathol*., **148**, 1089–1096.
24. Iannaccone,P.M. (1987) The study of mammalian organogenesis by mosaic pattern analysis. *Cell Differ*., **21**, 79–91.
25. Bannasch,P. and Zerban,H. (1990) Tumors of the liver. In Turusov,V.S. and Mohr,U. (eds), *Pathology of tumors in laboratory animals*, 2nd Edn, Vol. 1, *Tumors in the rat*, IARC Scientific Publishers, Lyon, France, pp. 199–240.
26. Turusov,V.S. and Takayama,S. (1979) Tumors of the liver. In Turusov,V.S. (ed.), *Pathology of tumors of the laboratory mouse*, Vol II., IARC Scientific Publishers, Lyon, France, pp. 193–233.
27. Bannasch,P. and Zerban,H. (1992) Predictive value of hepatic preneoplastic lesions as indicators of carcinogenic response. In Vainio,H., Magee,P.N., McGregor,D.B. and McMichael,A.J. (eds), *Mechanisms of carcinogenesis in risk identification*, IARC Scientific Publishers, Lyon, France, pp. 389–427.
28. Kaufmann,W.K., Zhang,Y. and Kaufman,D.G. (1992) Association between transforming growth factor-alpha and conversion of hepatocellular foci to neoplasms. *Carcinogenesis*, **13**, 1481–1483.
29. Tanno,S. and Ogawa,K. (1994) Abundant TGFα precursor and EGF receptor expression as a possible mechanism for the preferential growth of carcinogen-induced preneoplastic and neoplastic hepatocytes in rats. *Carcinogenesis*, **15**, 1689–1694.
30. Norstedt,G., Levinovitz,A., Möller,C., Eriksson,L.C. and Andersson,G. (1988) Expression of insulin-like growth factor I (IGF-I) and IGF-II mRNA during hepatic development, proliferation and carcinogenesis in the rat. *Carcinogenesis*, **9**, 209–213.
31. Cariani,E., Dubois,N., Lasserre,C., Briand,P. and Brechot,C. (1991) Insulin-like growth factor II (IGF-II) mRNA expression during hepatocarcinogenesis in transgenic mice. *J. Hepatol*., **13**, 220–226.
32. Yang,D. and Rogler,C.E. (1991) Analysis of insulin-like growth factor II (IGF-II) expression in neoplastic nodules and hepatocellular carcinomas

of woodchucks utilizing *in situ* hybridization and immunohistochemistry. *Carcinogenesis*, **12**, 1893–1901.
33. Maronpot,R.R., Montgomery,C.A., Boorman,G.A. and McConnell,E.E. (1986) National Toxicology Program nomenclature for hepatoproliferative lesions of rats. *Toxicol. Pathol.*, **14**, 263–273.
34. Rabes,H.N., Bucher,T., Hartman,A., Linke,I. and Dunnwald,M. (1982) Clonal origin of carcinogen-induced enzyme-deficient preneoplastic populations in the mouse. *Cancer Res.*, **42**, 3220–3227.
35. Weinberg,W.C., Berkwits,L. and Iannaccone,P.M. (1987) The clonal nature of carcinogen-induced altered foci of γ-glutamyl transpeptidase expression in rat liver. *Carcinogenesis*, **8**, 565–570.
36. Tsuji,S., Ogawa,K., Takasaka,H., Sonoda,T. and Mori,M. (1988) Clonal origin of γ-glutamyl transpeptidase-positive hepatic lesions induced by initiation-promotion in ornithine carbamoyltransferase mosaic mice. *Japan. J. Cancer Res.*, **79**, 148–151.
37. Weinberg,W.C. and Iannaccone,P.M. (1988) Clonality of preneoplastic liver lesions: histological analysis in chimeric rats. *J. Cell. Sci.*, **89**, 423–431.
38. Popper,H., Thung,S.N., McMahon,B.J., Lanier,A.P., Hawkins,I. and Alberts,S.B. (1988) Evolution of hepatocellular carcinoma associated with chronic hepatitis B virus infection in Alaskan Eskimos. *Arch. Pathol. Lab. Med.*, **112**, 498–504.
39. Unoura,M., Kaneko,S., Matsushita,E. *et al*. (1993) High-risk groups and screening strategies for early detection of hepatocellular carcinoma in patients with chronic liver disease. *Hepato-gastroenterol.*, **40**, 305–310.
40. Johnson,P.J. and Williams,R. (1987) Cirrhosis and the etiology of hepatocellular carcinoma. *J. Hepatol.*, **4**, 140–147.
41. Anthony,P.P., Ishak,K.G., Nayak,N.C., Paulsen,H.E., Scheuer,P.J. and Sobin,L.H. (1978) The morphology of cirrhosis. Recommendations on definition, nomenclature, and classification by a working group sponsored by the World Health Organization. *J. Clin. Pathol.*, **31**, 395–414.
42. Watanabe,S., Okita,K., Harada,T., Kodama,T., Numa,Y., Takemoto,T. and Takahashi,T. (1982) Morphologic studies of the liver dysplasia. *Cancer*, **51**, 2197–2205.
43. Thomas,R.M., Berman,J.J., Yetter,R.A., Moore,G.W. and Hutchins,G.M. (1992) Liver cell dysplasia: a DNA aneuploid lesion with distinct morphologic features. *Human Pathol.*, **23**, 496–503.
44. Yasui,H., Hino,O., Ohtake,K., Machinami,R. and Kitagawa,T. (1992) Clonal growth of hepatitis B virus-integrated hepatocytes in cirrhotic liver nodules. *Cancer Res.*, **52**, 6810–6814.
45. Aihara,T., Noguchi,S., Sasaki,Y., Nakano,H. and Imaoka,S. (1994) Clonal analysis of regenerative nodules in hepatitis C virus-induced liver cirrhosis. *Gastroenterology*, **107**, 1805–1811.
46. Lanier,A.P., McMahon,B.J., Alberts,S.R., Popper,H. and Heyward,W.L. (1987) Primary liver cancer in Alaskan natives 1980–1985. *Cancer*, **60**, 1915–1920.
47. Nakanuma,Y., Terada,T., Ueda,K., Terasaki,S., Nonomura,A. and Matsui,O. (1993) Adenomatous hyperplasia of the liver as a precancerous lesion. *Liver*, **13**, 1–9.
48. Ferrell,L.D., Crawford,J.M., Dhillon,A.P., Schever,P.J. and Nakanuma,Y. (1993) Proposal for standardized criteria for the diagnosis of benign, borderline, and malignant hepatocellular lesions arising in chronic advanced liver disease. *Am. J. Surg. Pathol.*, **17**, 1113–1123.
49. International Working Party (1995) Terminology of nodular hepatocellular lesions. *Hepatology*, **22**, 983–993.
50. Borzio,M., Bruno,S., Roncalli,M., Mels,G.C., Ramella,G., Borzio,F., Leandro,G., Servida,E. and Podda,M. (1995) Liver cell dysplasia is a major risk factor for hepatocellular carcinoma in cirrhosis: a prospective study. *Gastroenterology*, **108**, 812–817.
51. Stromeyer,F.W. and Ishak,K.G. (1981) Nodular transformation (nodular 'regenerative' hyperplasia) of the liver. A clinicopathologic study of 30 cases. *Human Pathol.*, **12**, 60–71.
52. Bannasch,P. and Klinge,O. (1971) Hepatocelluläre glykogenose und hepatombildung beim menschen. *Virchows Arch. A*, **352**, 157–164.
53. Cain,H. and Kraus,B. (1977) Entwicklungsstörungen der leber und leberkarzinom im sauglings-und kindesalter. *Dtsch. Med. Wschr.*, **102**, 505–509.
54. Fischer,G., Hartmann,H., Droese,M., Schaver,A. and Bock, K.W. (1986) Histochemical and immunohistochemical detection of putative preneoplastic foci in women after long-term use of oral contraceptives. *Virchows Arch. B*, **50**, 321–337.
55. Uchida,T., Miyata,H. and Shikata,T. (1981) Human hepatocellular carcinoma and putative precancerous disorders. *Arch. Pathol. Lab. Med.*, **105**, 180–186.
56. Thung,S.N. and Gerber,M.A. (1981) Enzyme patterns and marker antigens in nodular 'regenerative' hyperplasia of the liver. *Cancer*, **47**, 1796–1799.
57. Hirota,N., Hamazaki,M. and Williams, G.M. (1982) Resistance to iron accumulation and presence of hepatitis B surface antigen in preneoplastic and neoplastic lesions in human hemochromatotic livers. *Hepato-gastroenterol.*, **29**, 49–51.
58. Karhunen,D.J. and Penttilä,A. (1987) Preneoplastic lesions of human liver. *Hepato-gastroenterol.*, **34**, 10–15.
59. Bannasch,P., Jahn,U.-R. and Zerban,H. (1992) Preneoplastic lesions as early indicators of neoplastic development. In Bannasch,P. (ed.), *Cancer diagnosis. Early detection*. Springer–Verlag, Berlin, pp. 178–190.
60. Terada,T. and Nakanuma,Y. (1989) Iron-negative foci in siderotic macroregenerative nodules in human cirrhotic liver. A marker of incipient neoplastic lesions. *Arch. Pathol. Lab. Med.*, **113**, 916–920.
61. Terada,T. and Nakanuma,Y. (1989) Survey of iron-accumulative macroregenerative nodules in cirrhotic livers. *Hepatol.*, **10**, 851–854.
62. Govindarajan,S., Conrad,A., Lim,B., Valinluck,B., Kim,A.M. and Schmid,P. (1990) Study of preneoplastic changes in liver cells by immunohistochemical and molecular hybridization techniques. *Arch. Pathol. Lab. Med.*, **114**, 1042–1045.
63. Bannasch,P., Jahn,U.-R., Hoffmann,W., Pichlmayr,R. and Otto,G. (1996) Focal hepatic glycogenosis: a putative preneoplastic lesion associated with neoplasia and cirrhosis in explanted livers, submitted.
64. Deugnier,Y.M., Charalambous,P., LeQuilleuc,D., Turlin,B., Searle,J., Brissot,P., Powell,L.W. and Halliday,J.W. (1993) Preneoplastic significance of hepatic iron-free foci in genetic hemochromatosis: a study of 185 patients. *Hepatology*, **18**, 1363–1369.
65. Degunier,Y.M., Guyader,D., Crantock,L. *et al*. (1993) Primary liver cancer in genetic hematochromatosis: A clinical, pathological, and pathogenetic study of 54 cases. *Gastroenterology*, **104**, 228–234.
66. Terada,T., Kadoya,M., Nakamura,Y. and Matsui,O. (1990) Iron-accumulating adenomatous hyperplastic nodule with malignant focui in the cirrhotic liver. Histologic, quantitative iron, and magnetic resonance imaging *in vitro* studies. *Cancer*, **65**, 1994–2000.
67. Popper,H., Shih,J.W.-K., Gerin,J.L., Wong,D.C., Hoyer,B.H., London,W.T., Sly,D.L. and Purcell,R.H. (1981) Woodchuck hepatitis and hepatocellular carcinoma: correlation of histologic with virologic findings. *Hepatology*, **1**, 91–98.
68. Marion,P.L., VanDavelaar,M.J., Knight,S.S., Salazar,F.H., Garcia,G., Popper,H. and Robinson,W.S. (1986) Hepatocellular carcinoma in ground squirrels persistently infected with ground squirrel hepatitis virus. *Proc. Natl Acad. Sci.*, **83**, 4543–4546.
69. Toshkov,I., Hacker,H.J., Roggendorf,M. and Bannasch,P. (1990) Phenotypic patterns of preneoplastic and neoplastic hepatic lesions in woodchucks infected with woodchuck hepatitis virus. *J. Cancer Res. Clin. Oncol.*, **116**, 581–590.
70. Toshkov,I., Chisari,F.V. and Bannasch,P. (1994) Hepatic preneoplasia in hepatitis B virus transgenic mice. *Hepatology*, **20**, 1162–1172.
71. Office of Science and Technology Policy (1984) Chemical carcinogens; notice of review of the science and its associated principles. *Federal Reg.*, **49**, 21594–21661.
72. Sheridan,M.J. (1992) Analytical epidemiology: techniques to determine causal relationships, In Higginson,J., Muir,C.S. and Munoz,N. (eds), *Human cancer: Epidemiology and environmental causes.* Cambridge University Press, Cambridge, pp. 27–37.
73. International Agency for Research on Cancer (1994) *IARC Monographs on the evaluation of Carcinogenic risks to humans, Vol. 59, Hepatitis viruses*, IARC Scientific Publications, Lyon, France, p. 286.
74. International Agency for Research on Cancer. (1994) *IARC Monographs on the evaluation of carcinogenic risk to humans, Vol. 56, Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins*, IARC Scientific Publications, Lyon, France (see section on aflatoxins), pp. 245–395.
75. Peers,F., Bosch,X., Kaldor,J., Linsell,A. and Pluijmen,M. (1987) Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland. *Int. J. Cancer*, **39**, 545–553.
76. Ross,R.K., Yuan,J.-N., Yu,M.C., Wogan,G.N., Qian,G.-S., Tu,J.-T., Groopman,J.O., Gao,Y.-T. and Henderson,B.E. (1992) Urinary aflatoxin biomarkers and risk of hepatocellular cancer. *Lancet*, **339**, 943–946.
77. Qian,G.-S., Ross,R., Yu,M.C., Yuan,J.-M., Gao,Y.-T., Henderson,B.E., Wogan,G.N. and Groopman,J.D. (1994) A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiol. Biomark. Prev.*, **3**, 3–10.
78. Vainio,H. and Wilbourn,J. (1993) Cancer etiology: agents associated with human cancer. *Pharmac. Toxicol.*, **72**, S4–S11.
79. International Agency for Research on Cancer (1996) *IARC Monographs on the evaluation of Carcinogenic risk to humans, Vol. 66, Some medicinal drugs*, IARC Scientific Publications, Lyon, France, in press.
80. International Agency for Research on Cancer (1988) *IARC Monographs*

on the evaluation of Carcinogenic risks to humans, Vol. 44, Alcohol drinking, IARC Scientific Publications, Lyon, France, pp. 207–215.
81. Chen,C.-J., Chen,C.W., Wu,M.-M. and Kuo,T.-L. (1992) Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. *Brit. J. Cancer*, **66**, 888–892.
82. Andersson,M. and Storm,H.H. (1992) Cancer incidence among Danish Thoratrast-exposed patients. *J. Natl Cancer Inst.*, **84**, 1318–1325.
83. Andersson,M., Vyberg,M., Visfeldt,J., Carstensen,B. and Storm,H.H. (1994) Primary liver tumors among Danish patients exposed to Thoratrast. *Radiat. Res.*, **137**, 262–273.
84. Trichopoulos,D., MacMahon,B., Sparros,L. and Merikas,G. (1980) Smoking and hepatitis B-negative primary hepatocellular carcinomas. *J. Natl Cancer Inst.*, **65**, 111–114.
85. Hirayama,T. (1989) A large-scale cohort on risk factors for primary liver cancer, with special reference to the role of cigarette smoking. *Cancer Chemother. Pharmacol.*, **23**(Suppl.), S114–S117.
86. Chen,C.-J., Liang,K.-Y., Chang,A.-S., Chang,Y.-C., Lu,S.-N., Liaw,Y.-F., Chang,W.-Y., Sheen,M.-C. and Lin,T.-M. (1991) Effects of hepatitis B virus, alcohol drinking, cigarette smoking, and familial tendency on hepatocellular carcinoma. *Hepatol.*, **13**, 398–406.
87. Yu,M.C., Tong,M.J., Govindjaran,S. and Henderson,B.E. (1991) Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. *J. Natl Cancer Inst.* **83**, 1820–1826.
88. Yu,M.C., Chen,C.-J., Luo,J.-C., Brandt-Rauf,P.W., Carney,W.P. and Santella,R.M. (1994) Correlations of chronic hepatitis B virus and cigarette smoking with elevated expression of neu oncoprotein in the development of hepatocellular carcinoma. *Cancer Res.*, **54**, 5106–5110.
89. Austin,H., Delzell,E., Grufferman,S., Levine,R., Morrison,A.S., Stolley,P.D. and Cole,P. (1982) A case-control study of hepatocellular carcinoma and the hepatitis B virus, cigarette smoking, and alcohol consumption. *Cancer Res*, **46**, 962–966.
90. Tsukuma,H., Hiyama,T., Oshima,A. *et al*. (1990) A case-control study of hepatocellular carcinoma in Osaka, Japan. *Int. J. Cancer*, **45**, 231–236.
91. Tanaka,T., Hirohata,T., Takeshita,S., Hirohata,I., Koga,S., Sugimachi,K., Kanematsu,T., Ohryohji,F. and Ishibashi,H. (1992) Hepatitis B virus, cigarette smoking and alcohol consumption in the development of hepatocellular carcinoma: a case-control study in Fukuoka, Japan. *Int. J. Cancer*, **51**, 509–514.
92. Stemhagen,A., Slade,J., Altman,R. and Bill,J. (1983) Occupational risk factors and liver cancer. A retrospective case-control study of primary liver cancer in New Jersey. *Am. J. Epidemiol.*, **117**, 443–454.
93. Hardell,L., Bengtsson,N.O., Jonsson,U., Enksson,S. and Larsson,L.G. (1984) Etiological aspects on primary liver cancer with special regard to alcohol, organic solvents and acute intermittent porphyria—an epidemiological investigation. *Brit. J. Cancer*, **50**, 389–397.
94. Austin,H., Delzell,E., Grufferman,S., Levine,R., Morrison,A.S., Stolley,P.D. and Cole,P. (1987) Case-control study of hepatocellular carcinoma, occupation, and chemical exposure. *J. Occup. Med.*, **29**, 665–669.
95. Bulkley,J.D., Sather,H., Ruccione,K., Rogers,P.C.J., Haas,J.E., Henderson,B.E. and Hammond,G.D. (1989) A case-control study of risk factors for hepatoblastoma. A report from the Children's Cancer Study Group. *Cancer*, **64**, 1169–1176.
96. Cordier,S., Thuy,L.T.B., Verger,P., Bard,D., Dai,L.L., Larouze,B., Dazza,M., Quinh,H.T. and Abenham,L. (1993) Viral infections and chemical exposures as risk factors for hepatocellular carcinoma in Vietnam. *Int. J. Cancer*, **55**, 196–201.
97. The Selected Cancers Cooperative Study (1990) The association of selected cancers with service in the US military in Vietnam III. Hodgkin's disease, nasal cancer, nasopharyngeal cancer, and primary liver cancer. *Arch. Intern. Med.*, **150**, 2495–2505.
98. Niederau,C., Fischer,R., Sonnenberg,A., Stremmel,W., Trampisch,H.J. and Strohmeyer,G. (1985) Survival and causes of death in cirrhotic and noncirrhotic patients with primary hemochromatosis. *N. Engl. J. Med.*, **313**, 1256–1262.
99. Bianchi,L. (1993) Glycogen storage disease I and hepatocellular tumors. *Eur. J. Pediatr.*, **152**(Suppl. 1), 563–570.
100. Wilkinson,M.L., Portmann,B. and Williams,R. (1983) Wilson's disease and hepatocellular copper: possible protective role of copper. *Gut*, **24**, 767–771.
101. Eriksson,S., Carlson,J. and Velez,R. (1986) Risk of cirrhosis and primary liver cancer in alpha$_1$-antitrypsin deficiency. *N. Engl. J. Med.*, **314**, 736–739.
102. Russo,P. and O'Reagan,S. (1990) Visceral pathology of hereditary tyrosinemia type 1. *Am. J. Hum. Genet.* **47**, 317–324.
103. Weinstein,S., Scottalini,A.G., Loo,S.Y.T., Caldwell,P.C. and Bhagavan,N.V. (1985) Ataxia telangiectasia with hepatocellular carcinoma in a 15-year-old girl and studies of her kindred. *Arch. Pathol. Lab. Med.*, **109**, 1000–1004.
104. Jameson,C.F. (1989) Primary hepatocellular carcinoma in hereditary hemorrhagic telangiectasia. *Histopathol.*, **15**, 550–552.
105. Lithner,F. and Wetterberg,L. (1984) Hepatocellular carcinoma in patients with acute intermittent porphyria. *Acta Med. Scand.*, **215**, 271–274.
106. Kauppinen,R. and Mustajoki,P. (1988) Acute hepatic porphyria and hepatocellular cancer. *Brit. J. Cancer*, **57**, 117–120.
107. Siersma,P.D., tenKate,F.J.W., Mulder,P.G.H. and Wilson,J.H.P. (1992) Hepatocellular carcinoma in porphyria cutanea tarda, frequency and factors in its occurrence. *Liver*, **12**, 58–61.
108. Coleman,W.B. and Tsongalis,G.J. (1995) Multiple mechanisms account for genomic instability and molecular mutation in neoplastic transformation. *Clin. Chem.*, **41**, 644–657.
109. Massey,T.E., Stewart,R.K., Daniels,J.M. and Liu,L. (1995) Biochemical and molecular aspects of susceptibility to aflatoxin B_1 carcinogenicity. *Proc. Soc. Exp. Biol. Med.*, **208**, 213–227.
110. Yu,M.-W., Gladek-Yarborough,A., Chiamprasert,S., Santella,R.M., Liaw,Y.-F. and Chen,C.-J. (1995) Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. *Gastroenterology*, **109**, 1266–1273.
111. Wild,C.P., Hasegawa,R., Berraud,L., Chutimatdewin,S., Chapot,B., Ito,N. and Montesano,R. (1996) Aflatoxin-albumin adducts, a basis for competitive carcinogenesis between animals and humans. *Cancer Epid. Biomark. Prev.*, **5**, 179–189.
112. McGlynn,K.A., Rosvold,E.A., Lustbader,E.D. *et al*. (1995) Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B_1. *Proc. Natl Acad. Sci. USA*, **92**, 2384–2387.
113. Alberts,S.R., Lanier,A.P., McMahon,B.J., Harpster,A., Bulkow,L.R., Heyward,W.L. and Murray,C. (1991) Clustering of hepatocellular carcinoma in Alaska native families. *Genet. Epidemiol.*, **8**, 127–139.
114. Shen,F.-M., Lee,M.K., Gong,H.-M., Cai,X.-Q. and King,M.C. (1991) Complex segregation analysis of hepatocellular carcinoma in Chinese families, interaction of inherited susceptibility and hepatitis B viral infection. *Am. J. Hum. Genet.*, **49**, 88–93.
115. Harris,C.C. and Sun,T.-T. (1984) Multifactoral etiology of human liver cancer. *Carcinogenesis*, **5**, 697–701.
116. Stroffolini,T., Chiaramonte,M., Tiribelli,C. *et al*. (1992) Hepatitis C virus infection, HBsAg carrier state and hepatocellular carcinoma, relative risk and population attributable risk from a case-control study in Italy. *J. Hepatol.*, **16**, 360–363.
117. Benvegnù,L., Fattovich,G., Noventa,F., Tremolada,F., Chemello,L., Cecchetto,A. and Alberti,A. (1994) Concurrent hepatitis B and C virus infection and risk of hepatocellular carcinoma in cirrhosis. *Cancer*, **74**, 2442–2448.
118. Nalpas,B., Feitelson,M., Bréchot,C. and Rubin,E. (1995) Alcohol, hepatotropic viruses and hepatocellular carcinoma. *Alcoholism Clin. Exp. Res.*, **19**, 1089–1095.
119. Noda,K., Yoshihara,H., Suzuki,K., Yamada,Y., Kasahara,A., Hayashi,N., Fusamoto,H. and Kamada,T. (1996) Progression of type C chronic hepatitis to liver cirrhosis and hepatocellular carcinoma–its relationship to alcohol drinking and age of transfusion. *Alcohol. Clin. Exp. Res.*, **20**, 95A–100A.
120. Ikeda,K., Saitoh,S., Koida,I., Arase,Y., Tsubota,A., Chayama,K., Kumada,H. and Kawanishi,J. (1993) A multivariate analysis of risk factors for hepatocellular carcinogenesis, a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology*, **18**, 47–53.
121. Tanaka,K., Hirohata,T., Koga,S. *et al*. (1991) Hepatitis C and B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer Res.*, **51**, 2842–2847.
122. Rocchi,E., Gibertini,P., Caranelli,M., Pietrangelo,A., Jenson,J. and Ventura,E. (1986) Hepatitis B virus infection in porphyria cutanea tarda. *Liver*, **6**, 153–157.
123. Gold,L.S., Manley,N.B., Slone,T.H., Garfinkel,G.B., Ames,B.N., Rohrbach,L., Stern,B.R. and Chow,K. (1995) Sixth plot of the Carcinogenic Potency Database, results of animal bioassays published in the general literature 1989 to 1990 and by the National Toxicology Program 1990 to 1993. *Environ. Health Perspect.*, **103**(Suppl. 8), 3–123.
124. Gold,L.S., Slone,T.H., Stern,B.B. and Bernstein,L. (1993) Comparison of target organs of carcinogenicity for mutagenic and non-mutagenic chemicals. *Mutat. Res.*, **186**, 75–100.
125. Ward,J.M., Fox,J.G., Anver,M.R. *et al*. (1994) Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel *Helicobacter* species. *J. Natl Cancer Inst.*, **86**, 1222–1227.

126. International Agency for Research on Cancer (1994) *IARC Monographs on the evaluation of carcinogenic risks to humans, Vol. 61, Schistosomes, liver flukes and Helicobacter pylori*. IARC Scientific Publications, Lyon, France, pp. 127–240.
127. Sakamoto,H., Sawada,N., Kamimura,Y., Enomoto,K. and Mori,M. (1993) High sensitivity of LEC rats with chronic hepatitis to hepatocarcinogenesis, decreases in unscheduled and replicative DNA synthesis of the hepatocytes. *Japan. J. Cancer Res.*, **84**, 966–971.
128. Muramatsu,Y., Yamada,T., Miura,M., Sakai,T., Suzuki,Y., Serikawa,T., Tanzi,R. and Matsumoto,K. (1994) Wilson's disease gene is homologous to hts causing abnormal copper transport in Long-Evans Cinnamon rats. *Gastroenterology*, **107**, 1189–1192.
129. Cox,D.W. (1985) Genes of the copper pathway. *Am. J. Hum. Genet*., **56**, 828–830.
130. Grompe,M., Lindstedt,S., Al-Dhalimy,M., Kenneway,N.G., Papaconstantinou,J., Torres-Ramos,C.A., Ou,C.-N. and Finegold,M. (1995) Pharmacological correction of neonatal lethal hepatic dysfunction in a murine model of tyrosinemia type 1. *Nature Genet*., **10**, 453–460.
131. Sher,S.P., Jensen,R.D. and Bokelman,D.L. (1982) Spontaneous tumors in control F344 and Charles River-CD rats and Charles River CD-1 and B6C3HF1 mice. *Toxicol. Lett.*, **11**, 103–110.
132. Rao,G.N., Birnbaum,L.S., Collins,J.J., Tennant,R.W. and Skow,L.L. (1988) Mouse strains for chemical carcinogenicity studies, overview of a workshop. *Fund. Appl. Toxicol*., **10**, 385–394.
133. Dragani,T.A., Manenti,G., Gariboldi,M., Falvella,F.S., Pierotti,M.A. and DellaPorta,G. (1992) Genetics of hepatocarcinogenesis in mouse and man. In Zervos,C. (ed.), *Oncogene and transgenics correlates of cancer risk assessments*, Plenum Press, New York, pp. 67–80.
134. Drinkwater,N.R., Hanigan,M.H. and Kemp,C.J. (1989) Genetic determinants of hepatocarcinogenesis in the B6C3F1 mouse. *Toxicol. Lett*., **49**, 255–265.
135. Gariboldi,M., Manenti,G., Canzian,F., Falvella,F.S., Pierotti,M.A., DellaPorta,G., Sinelli,G. and Dragani,T.A. (1993) Chromosome mapping of murine liver tumor loci to liver carcinogenesis. *Cancer Res*., **53**, 209–211.
136. Manenti,G., Binelli,G., Gariboldi,M., Canzian,F., DeGregorio,L., Falvella,F.S., Dragani,T.A. and Pierotti,M.A. (1994) Multiple loci affect genetic predisposition to hepatocarcinogenesis in mice. *Genomics*, **23**, 118–124.
137. Lee,G.-H. and Drinkwater,N.R. (1995) The *Hcr* (hepatocarcinogen resistance) loci of DBA/2J mice partially suppress phenotypic expression of HCS (hepatocarcinogen sensitivity) loci of C3H/HeJ mice. *Carcinogenesis*, **16**, 1993–1996.
138. Purchase,I.F.H. (1980) Inter-species comparisons of carcinogenicity. *Brit. J. Cancer*, **41**, 454–468.
139. Ashby,J. and Tennant,R.W. (1988) Chemical structure, *Salmonella* mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the US NCI/NTP. *Mutat. Res.*, **204**, 17–115.
140. Gold,L.S., Manley,N.B. and Ames,B.N. (1992) Extrapolation of carcinogenicity between species, qualitative and quantitative factors. *Risk Anal*., **12**, 579–588.
141. Vancutsem,P.M., Lazarus,P. and Williams,G.M. (1994) Frequent and specific mutations of the rat *p53* gene in hepatocarcinomas induced by tamoxifen. *Cancer Res.*, **54**, 3864–3867.
142. Ennever,F.K., Noonan,T.J. and Rosenkranz,H.S. (1987) The predictivity of animal bioassays and short-term genotoxicity tests for carcinogenicity and non-carcinogenicity in humans. *Mutagenesis*, **2**, 73–78.
143. Marselos,M. and Vainio,H. (1991) Carcinogenic properties of pharmaceutical agents evaluated in the IARC Monographs programme. *Carcinogenesis*, **12**, 1751–1766.
144. Yager,J.D., Zurlo,J. and Ni,N. (1991) Sex hormone and tumor promotion in the liver. *Proc. Soc. Exp. Biol. Med*., **198**, 667–674.
145. Schulte-Hermann,R. (1985) Tumor promotion in the liver. *Arch. Toxicol*., **57**, 147–158.
146. Peraino,C. (1981) Initiation and promotion of liver tumorigenesis. *Natl Cancer Inst. Monogr.*, **58**, 55–61.
147. White,S.J., McLean,A.E.M. and Howland,C. (1979) Anticonvulsant drugs and cancer, a cohort study in patients with severe epilepsy. *Lancet*, **2**, 458–461.
148. Shirts,S.B., Annegers,J.F., Hauser,W.A. and Kurland,L.T. (1986) Cancer incidence in a cohort of patients with seizure disorders. *J. Natl Cancer Inst*., **77**, 83–87.
149. Olsen,J.H., Boice,J.D., Jensen,J.P. and Fraumeni,J.F. (1989) Cancer among epileptic patients exposed to anticonvulsant drugs. *J. Natl Cancer Inst*., **81**, 803–808.
150. Porta,E.A., Markell,N. and Dorado,R.D. (1985) Chronic alcoholism enhances hepatocarcinogenecity of diethylnitrosamine in rats fed a marginally methyl-deficient diet. *Hepatology*, **5**, 1120–1125.
151. Takada,A., Nei,J. Takase,S. and Matsuda,Y. (1986) Effects of ethanol on experimental hepatocarcinogenesis. *Hepatology*, **6**, 65–72.
152. Yamagiwa,K., Mizumoto,R., Higashi,S., Kato,H., Tomida,T., Uehara, S.-I., Tanigawa,K., Tanaka,M. and Ishida,N. (1994) Alcohol ingestion enhances hepatocarcinogenesis induced by synthetic estrogen and progestin in the rat. *Cancer Detect. Prev.*, **18**, 103–114.
153. Froment,O., Boivin,S., Barbin,A., Bancel,B., Trepo,C. and Marion,M.-J. (1994) Mutagenesis of *ras* proto-oncogenes in rat liver tumors induced by vinyl chloride. *Cancer Res.*, **54**, 5340–5345.
154. Marion,M.J., Froment,O. and Trepo,C. (1991) Activation of Ki-*ras* gene by point mutation in human liver angiosarcoma associated with vinyl chloride exposure. *Mol. Carcinogenesis*, **4**, 450–454.
155. Zerban,H., Radig,S., Kopp-Schneider,A. and Bannasch,P. (1994) Cell proliferation and cell death (apoptosis) in hepatic preneoplasia and neoplasia are closely related to phenotypic cellular diversity and instability. *Carcinogenesis*, **15**, 2467–2473.
156. Farber,E. and Sarma,D.S.R. (1987) Hepatocarcinogenesis, a dynamic cellular perspective. *Lab. Invest*., **56**, 4–22.
157. Schulte-Hermann,R., Bursh,W. and Grasl-Kraupp,B. (1995) Active cell death (apoptosis) in liver biology and disease. *Prog. Liver Dis*., **13**, 1–35.
158. Sell,S. and Dunsford,H.A. (1989) Evidence for the stem cell origin of hepatocellular carcinoma and cholangiocarcinoma. *Am. J. Pathol*., **134**, 1347–1361.
159. Fu,X., Su,X., Lee,C.Y., Hintz,R., Biempica,L., Snyder,R. and Rogler,C.E. (1988) Insulin-like growth factor II expression and oval cell proliferation associated with hepatocellular carcinogenesis in woodchuck hepatitis virus carriers. *J. Virol*., **62**, 3422–3438.
160. Tsao,M.-S. and Grisham,J.W. (1987) Hepatocarcinomas, cholangiocarcinomas and hepatoblastomas produced by chemically transformed cultured rat liver epithelial cells. A light and electron microscopic study. *Am. J. Pathol*., **127**, 168–181.
161. Hsia,C.C., Evarts,R.P., Nakatsukasa,H., Marsden,E.R. and Thorgeirsson,S.S. (1992) Occurrence of oval-type cells in hepatitis B virus-associated human hepatocarcinogenesis. *Hepatology*, **16**, 1327–1333.
162. Hsia,C.C., Thorgeirsson,S.S. and Tabor,E. (1994) Expression of hepatitis B surface and core antigens and transforming growth factor-α in 'oval cells' of the liver in patients with hepatocellular carcinoma. *J. Med. Virol*., **43**, 216–221.
163. Hoel,D.G., Haseman,J.R., Hogan,M.D., Huff,J. and McConnell,E.E. (1988) The impact of toxicity on carcinogenicity studies, implications for risk assessment. *Carcinogenesis*, **9**, 2045–2052.
164. Tennant,R.W., Elwell,M.R., Spalding,J.W. and Griesemer,R.A. (1991) Evidence that toxic injury is not always associated with induction of chemical carcinogenesis. *Mol. Carcinogenesis*., **4**, 420–440.
165. Hartwell,L.H. and Kastan,M.B. (1994) Cell cycle control and cancer. *Science*, **266**, 1821–1828.
166. Fausto,N. (1991) Growth factors in liver development, regeneration and carcinogenesis. *Prog. Growth Factor Res*., **3**, 219–234.
167. Miller,R.T., Cattley,R.C., Marsman,D.S., Lyght,O. and Popp,J.A. (1995) TGFα is differentially expressed in liver foci induced by diethylnitrosamine initiation and peroxisome proliferator promotion. *Carcinogenesis*, **16**, 77–82.
168. Dragan,Y., Teeguarden,J., Campbell,H., Hsia,S. and Pitot,H. (1995) The quantitation of altered hepatic foci during multistage hepatocarcinogenesis in the rat, transforming growth factor α expression as a marker of the stage of progression. *Cancer Lett*., **93**, 73–83.
169. Jirtle,R.L. and Meyer,S.A. (1991) Liver tumor promotion, effect of phenobarbital on EGF and protein kinase C signal transduction and transforming growth factor -β1 expression. *Digest. Dis. Sci*., **36**, 659–688.
170. Jhappan,C., Stahle,C., Harkins,R.N., Fausto,N., Smith,G.H. and Merlino,G.T. (1990). TGFα overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. *Cell*, **61**, 1137–1146.
171. Russell,W.E., Kaufmann,W.K., Sitaric,S., Luetteke,N.C. and Lee,D.C. (1996) Liver regeneration and hepatocarcinogenesis in transforming growth factor-α-targetted mice. *Mol. Carcinogenesis*, **15**, 183–189.
172. Castilla,A., Prieto,J. and Fausto,N. (1991) Transforming growth factor β1 and α in chronic liver disease, effect of interferon alfa therapy. *N. Engl. J. Med*., **324**, 933–940.
173. Collier,J.D., Guo,K., Gullick,W.J., Bassendine,M.F. and Burt,A.D. (1993) Expression of transforming growth factor alpha in human hepatocellular carcinoma. *Liver*, **13**, 151–155.
174. Park,B.C., Hoh,M.H. and Seo,J.H. (1995) Differential expression of transforming growth factor α and insulin-like growth factor II in chronic

active hepatitis B, cirrhosis and hepatocellular carcinoma. *J. Hepatol*., **22**, 286–294.
175. Hsia,C.C., Axiotis,C.A., DiBisceglie,A.M. and Tabor,E. (1992) Transforming growth factor-alpha in human hepatocellular carcinoma and coexpression with heptitis B surface antigen in adjacent liver cancer. *Cancer*, **70**, 1049–1056.
176. Morimitsu,Y., Hsia,C.C., Kojiro,M. and Tabor,E. (1995) Nodules of less-differentiated tumor within or adjacent to hepatocellular carcinoma, relative expression of transforming growth factor-α and its receptor in the different areas of tumor. *Human Pathol*., **26**, 1126–1132.
177. Yeh,Y.-C., Tsai,J.-F., Chuang,L.-Y., Yeh,H.-W., Tsai,J.-H., Florine,L. and Tam,J.P. (1987) Elevation of transforming growth factor α and its relationship to the epidermal growth factor and α-fetoprotein levels in patients with hepatocellular carcinoma. *Cancer Res.*, **47**, 896–901.
178. Chuang, L.-Y., Tsai,J.-H., Yeh,Y.-C., Chang,C.-C., Yeh,H.-W., Guh,Y.-Y. and Tsai,J.-F. (1991) Epidermal growth factor-related transforming growth factors in the urine of patients with hepatocellular carcinoma. *Hepatology*, **13**, 1112–1116.
179. Tomiya,T. and Fujiwara,K. (1996) Serum transforming growth factor α as a marker of hepatocellular carcinoma complicating cirrhosis. *Cancer*, **77**, 1056–1060.
180. Ueno,T., Takahashi,K., Matsuguchi,T., Ikejiri,K., Endo,H. and Yamamoto,M. (1988) Reactivation of rat insulin-like growth factor II gene during hepatocarcinogenesis. *Carcinogenesis*, **9**, 1779–1783.
181. Schirmacher,P., Held,W.A., Yang,D., Chisari,F.V., Rustum,Y. and Rogler, C.E. (1992) Reactivation of insulin-like growth factor II during hepatocarcinogenesis suggests a role in malignant growth. *Cancer Res.*, **52**, 2549–2556.
182. Jirtle,R.L., Hankins,R., Reisenbacher,H. and Boyer,I.J. (1994) Regulation of mannose 6-phosphate/insulin-like growth factor-II receptors and transforming growth factor beta during liver tumor promotion with phenobarbital. *Carcinogenesis*, **15**, 1473–1478.
183. Rumsby,P.C., Davies,M.J., Price,R.J. and Lake,B.G. (1994) Effect of some peroxisome proliferators on transforming growth factor-β_1 gene expression and insulin-like growth factor II/mannose-6-phosphate receptor gene expression in rat liver. *Carcinogenesis*, **15**, 419–421.
184. Cariani,E., Lasserre,C., Seurin,D., Hamelin,B., Kemeny,F., Franco,D., Czech,M.P., Ullrich,A. and Brechot,C. (1988) Differential expression of insulin-like growth factor II in RNA in human primary liver cancers, benign liver tumors and liver cirrhosis. *Cancer Res.*, **48**, 6844–6849.
185. Lamas,E., LeBail,B., Housset,C., Boucher,O. and Brechot,C. (1991) Localization of insulin-like growth factor-II and hepatitis B virus mRNAs and proteins in human hepatocellular carcinomas. *Lab. Invest*., **64**, 98–104.
186. Fiorentino,M., Grigoni,W.F., Baccarini,P., D'Errico,A., DeMitri,M.S., Pisi,E. and Mancini,A.M. (1994) Different *in situ* expression of insulin-like growth factor type II in hepatocellular carcinoma, an *in situ* hybridization and histochemical study. *Diag. Mol. Pathol*., **3**, 59–65.
187. D'Arville,C.N., Nouri-Aria,K.T., Johnson,P. and Williams,R. (1991) Regulation of insulin-like growth factor II gene expression by hepatitis B virus in hepatocellular carcinoma. *Hepatology*, **13**, 310–315.
188. Cariani,E., Lasserre,C., Kemeny,F., Franco,D. and Brechot,C. (1991) Expression of insulin-like growth factor II, α-fetoprotein and hepatitis B virus transcripts in human primary liver cancer. *Hepatology*, **13**, 644–649.
189. Daughaday,W.H., Wu,J.-C., Lee,S.-D. and Kapadia,M. (1990) Abnormal processing of pro-IGF-II in patients with hepatoma and some hepatitis B virus antibody-positive asymptomatic individuals. *J. Lab. Clin. Med*., **115**, 555–562.
190. Cariani,E., Seurin,D., Lasserre,C., Franco,D., Binoux,M. and Brechot,C. (1990) Expression of insulin-like growth factor II (IGF-II) in human primary liver cancer, mRNA and protein analysis. *J. Hepatol.*, **11**, 226–231.
191. Akmal,S.N., Yun,K., MacLay,J., Higami,Y. and Ikeda,T. (1995) Insulin-like growth factor 2 and insulin-like growth factor binding protein 2 expression in hepatoblastoma. *Human Pathol*., **26**, 851–858.
192. Davies,S.M. (1993) Maintenance of genomic imprinting at the IGF2 locus in hepatoblastoma. *Cancer Res.*, **53**, 4781–4783.
193. Ranier,S., Dobry,C.J. and Feinberg,A.P. (1995) Loss of imprinting in hepatoblastoma. *Cancer Res.*, **55**, 1836–1838.
194. Li,X., Adam,G., Cui,H., Sandstedt,B., Ohlsson,R. and Ekström,T.J. (1995) Expression, promoter, usage and imprinting status of insulin-like growth factor II (IGF2) in human hepatoblastomas, uncoupling of IGF2 and H19 imprinting. *Oncogene*, **11**, 221–229.
195. Sue,S.R., Chari,R.S., Kong,F.-M., Mills,J.J., Fine,R.L., Jirtle,R.L. and Meyers,W.C. (1995) Transforming growth factor beta receptors and mannose 6-phosphate/insulin-like growth factor-II receptor expression in human hepatocellular carcinomas. *Ann. Surg*., **222**, 171–178.
196. DeSouza,A.T., Hankins,G.R., Washington,M.K., Fine,R.L., Orton,T.C. and Jirtle,R.L. (1995) Frequent loss of heterozygosity on 6q at the mannose 6-phosphate receptor/insulin-like growth factor II receptor locus in human hepatocellular tumors. *Oncogene*, **10**, 1725–1729.
197. DeSouza,A.T., Hankins,G.R., Washington,M.K., Orton,T.C. and Jirtle,R.L. (1995) M6P/IGF2R gene is mutated in human hepatocellular carcinoma with loss of heterozygosity. *Nature Genet*., **11**, 447–449.
198. Rogler,C.E., Yang,D., Rossetti,L., Donohoe,J., Alt,E., Chang,C.J., Rosenfeld,R., Neely,K. and Hintz,R. (1994) Altered body composition and increased frequency of various malignancies in insulin-like growth factor-II transgenic mice. *J. Biol. Chem*., **269**, 13779–13784.
199. Norgaard,P., Hougaard,S., Poulsen,H.S. and Spang-Thomsen,M. (1995) Transforming growth factor β and cancer. *Cancer Treat. Rev.*, **21**, 367–403.
200. Reisenbichler,H., Chari,R.S., Boyer,I.J. and Jirtle,R.L. (1994) Transforming growth factor-β receptors type I, II and III in phenobarbital-promoted rat liver tumors. *Carcinogenesis*, **15**, 2763–2767.
201. Ito,N., Kawata,S., Tamura,S., Takaishi,K., Shirai,Y., Kiso,S., Yabuuchi,I., Matsuda,Y., Nichioka,M. and Tarui,S. (1991) Elevated levels of transforming growth factor β messenger RNA and its polypeptide in human hepatocellular carcinoma. *Cancer Res.*, **51**, 4080–4083.
202. Shirai,Y., Kawata,S., Tamura,S., Ito,N., Tsushima,H., Takaishi,K., Kiso,S. and Matsuzawa,Y. (1994) Plasma transforming growth factor-β1 in patients with hepatocellular carcinoma. Comparison with chronic liver diseases. *Cancer*, **73**, 2275–2279.
203. Strom,S.C. and Faust,J.B. (1990) Oncogene activation and hepatocarcinogenesis. *Pathobiology*, **58**, 153–167.
204. Deguchi,T. and Pitot,H.C. (1995) Expression of *c-myc* in altered hepatocyte foci induced in rats by various single doses of diethylnitrosamine and promotion by 0.05% phenobarbital. *Mol. Carcinogenesis*, **14**, 152–159.
205. Himeno,Y., Fukuda,Y., Hatanaka,M. and Imura,H. (1989) Expression of oncogenes during rat chemical hepatocarcinogenesis. *Japan. J. Cancer Res.*, **80**, 737–742.
206. Porsch-Hällstrom,I., Blanck,A., Ericksson,L.C. and Gustafsson,J.-A. (1989) Expression of the c-*myc*, c-*fos* and c-*ras*^{Ha} proto-oncogenes during sex-differentiated rat liver carcinogenesis in the resistant hepatocyte model. *Carcinogenesis*, **10**, 1793–1800.
207. Galand,P., Jacobovitz,D. and Alexandre,K. (1988) Immunohistochemical detection of c-Ha-*ras* oncogene *p21* product in pre-neoplastic and neoplastic lesions during hepatocarcinogenesis in rats. *Int. J. Cancer*, **41**, 155–161.
208. Sills,R.C., Goldsworthy,T.L. and Sleight,S.D. (1994) Tumor-promoting effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and phenobarbital in initiated weanling Sprague–Dawley rats, a quantitative, phenotypic and *ras* p21 protein study. *Toxicol. Pathol*., **22**, 270–281.
209. Suchy,B.K., Sarafoff,M., Kerler,R. and Rabes,H.M. (1989) Amplification, rearrangements and enhanced expression of c-*myc* in chemically induced rat liver tumors *in vivo* and *in vitro*. *Cancer Res.*, **49**, 6781–6787.
210. Chandar,N., Lombardi,B. and Locker,J. (1989) c-*myc* gene amplification during hepatocarcinogenesis by a choline-devoid diet. *Proc. Natl Acad. Sci. USA*, **86**, 2703–2707.
211. Bhave,M.B., Wilson,M.J. and Poirier,L.A. (1988) c-H-*ras* and c-K-*ras* gene hypomethylation in the livers and hepatomas of rats fed methyl-deficient, amino acid-defined diets. *Carcinogenesis*, **9**, 343–348.
212. Ray,J.S., Harbison,M.L., McClain,R.M. and Goodman,J.I. (1994) Alterations in the methylation status and expression of the *raf* oncogene in phenobarbital-induced and spontaneous B6C3F1 mouse liver tumors. *Mol. Carcinogenesis*, **9**, 155–166.
213. Counts,J.L. and Goodman,J.I. (1993) Comparative analysis of the 5′ flanking region of Ha-*ras* in B6C3F**1**, C3H/He and C57BL/6 mouse liver. *Cancer Lett*., **75**, 129–136.
214. Hansen,L.J., Tennant,B.C., Seeger,C. and Ganem,D. (1993) Differential activation of *myc* gene family members in hepatic carcinogenesis by closely related hepatitis B viruses. *Mol. Cell. Biol*., **13**, 659–667.
215. Yang,D., Alt,E. and Rogler,C.E. (1993) Coordinate expression of N-*myc* 2 and insulin-like growth factor II in precancerous altered hepatic foci in woodchuck hepatitis virus carriers. *Cancer Res.*, **53**, 2020–2027.
216. Gu,J.-R., Hu,L.-F., Cheng,Y.-C. and Wan,D.-F. (1986) Oncogenes in human primary hepatic cancer. *J. Cell. Physiol*., **4**(Suppl.), 13–20.
217. Himeno,Y., Fukuda,Y., Hatanaka,M. and Imura,H. (1988) Expression of oncogenes in human liver disease. *Liver*, **8**, 208–212.
218. Zhang,X.-K., Huang,D., Qiu,D.-K. and Chiu,J. (1990) The expression of c-*myc* and c-N-*ras* in human cirrhotic livers, hepatocellular carcinomas and liver tissue surrounding the tumors. *Oncogene*, **5**, 909–914.
219. Abou-Elella,A., Gramlich,T., Fritsch,C. and Gansler,T. (1996) c-*myc* amplification in hepatocellular carcinoma predicts unfavorable prognosis. *Mod. Pathol*., **9**, 95–98.
220. Kaneko,Y., Shibuya,M., Nakayama,T., Hayashida,N., Toda,G., Endo,Y.,

Oka,H. and Oda,T. (1985) Hypomethylation of c-*myc* and epidermal growth factor receptor genes in human hepatocellular carcinoma and fetal liver. *Japan. J. Cancer Res.*, **76**, 1136–1140.
221. Taylor,J.A., Bell,D.A. and Nagorney,D. (1993) L-*myc* proto-oncogene alleles and susceptibility to hepatocellular carcinoma. *Int. J. Cancer*, **54**, 927–930.
222. Nonomura,A., Ohta,G., Hayashi,M., Izumi,R., Watanabe,K., Takayanagi,N., Mizukami,Y. and Matsubara,F. (1987) Immunohistochemical detection of *ras* oncogene *p21* product in liver cirrhosis and hepatocellular carcinoma. *Am. J. Gastroenterol*., **82**, 512–518.
223. Radosevich,J.A., Gould,K.A., Koukoulis,G.K., Haines,G.K., Rosen,S.T., Lee,I. and Gould,V.E. (1993) Immunolocalization of *ras* oncogene *p21* in human liver diseases. *Ultrastruct. Pathol*., **17**, 1–18.
224. Murakami,H., Sanderson,N.D., Nagy,P., Marino,P., Merlino,G. and Thorgeirsson,S.S. (1993) Transgenic mouse model for synergistic effects of nuclear oncogenes and growth factors in tumorigenesis, interaction of c-*myc* and transforming growth factor α in hepatic oncogenesis. *Cancer Res.*, **53**, 1719–1723.
225. deThé,H., Marchio,A., Tiollais,P. and Dejean,A. (1987) A novel steroid thyroid hormone receptor-related gene inappropriately expressed in human hepatocellular carcinoma. *Nature*, **330**, 667–670.
226. Boix,L., Rosa,J.L., Ventura,F., Castells,A., Bruix,J., Rodés,J. and Bartrons,R. (1994) c-*met* RNA overexpression in human hepatocellular carcinoma. *Hepatology*, **19**, 88–91.
227. Suzuki,K., Hayashi,N., Yamada,Y. *et al*. (1994) Expression of the c-*met* protooncogene in human hepatocellular carcinoma. *Hepatology*, **20**, 1231–1236.
228. Grigoni,W.F., Fiorentino,M., D'Errico,A., Ponzetto,A., Crepaldi,T., Prat,M. and Comoglio, P.M. (1995) Overexpression of c-*met* protooncogene product and raised Ki67 index in hepatocellular carcinoma with respect to benign liver conditions. *Hepatology*, **21**, 1543–1546.
229. Brunt,E.M. and Swanson,P.E. (1992) Immunoreactivity for c-erbB-2 oncopeptide in benign and malignant diseases of the liver. *Am. J. Clin. Pathol*., **97**(Suppl.1), s53–s61.
230. Luo,J.-C., Yu,M.-W., Chen,C.-J., Santella,R.M., Carney,W.P. and Brandt-Rauf,P.W. (1993) Serum c-*erb*B-2 oncopeptide in hepatocellular carcinogenesis. *Med. Sci. Res*., **21**, 305–307.
231. Sever,C. and Locker,J. (1991) Expression of retinoic acid α and β receptor genes in liver and hepatocellular carcinoma. *Mol. Carcinogenesis*, **4**, 138–144.
232. Huang,L.-R. and Hsu,H.-C. (1995) Cloning and expression of *CD24* gene in human hepatocellular carcinoma, a potential early tumor marker gene correlates with *p53* mutation and tumor differentiation. *Cancer Res.*, **55**, 4717–4721.
233. Zhang,Y.J., Tiang,W., Chen,C.J., Lee,C.S., Kahn,S.M., Santella,R.M. and Weinstein,B.I. (1993) Amplification and overexpression of cyclin D1 in human hepatocellular carcinoma. *Biochem. Biophys. Res. Commun*., **196**, 1010–1012.
234. Nishida,N., Fukuda,Y., Komeda,T., Kita,R., Sando,T., Nakao,K., Ikenaga,M. and Ishizaki,K. (1994) Amplification and overexpression of cyclin D1 gene in aggressive human hepatocellular carcinoma. *Cancer Res.*, **54**, 3107–3110.
235. Maronpot,R.R., Fox,T., Malarkey,D.E. and Goldsworthy,T.L. (1995) Mutations in the *ras* proto-oncogene, clues to etiology and molecular pathogenesis of mouse liver tumors. *Toxicol*., **101**, 125–156.
236. McMahon,G., Davis,E.F., Huber,J., Kim,Y. and Wogan,G.N. (1990) Characterization of c-Ki-*ras* and N-*ras* oncogenes in aflatoxin B_1-induced rat liver tumors. *Proc. Natl Acad. Sci. USA*, **87**, 1104–1108.
237. Ostermayr,R., Suchy,B. and Rabes,H.M. (1991) Carcinogen-induced liver tumors of Wistar rats, absence of activated *ras* genes and of N-*ras*C. *J. Cancer Res., Clin. Oncol*., **117**, 381–384.
238. Tokushima,Y., Fukuda,I. and Ogawa,K. (1994) Absence of *p53* mutations and various frequencies of Ki-*ras* exon 1 mutations in rat hepatic tumors induced by different carcinogens. *Mol. Carcinogenesis*, **10**, 45–51.
239. Sinha,S., Webber,C., Marshall,C.J., Knowles,M.A., Proctor,A., Barass,N.C. and Neal,G.E. (1988) Activation of *ras* oncogenes in aflatoxin-induced rat liver carcinogenesis. *Proc. Natl Acad. Sci. USA*, **85**, 3673–3677.
240. Stowers,S.J., Wiseman,R.W., Ward,J.M., Miller,E.C., Miller,J.A., Anderson,M.W. and Eva,A. (1988) Detection of activated proto-oncogenes in N-nitrosodiethylamine-induced liver tumors, a comparison between B6C3F1 mice and Fischer 344 rats. *Carcinogenesis*, **9**, 271–276.
241. Watatani,M., Perantoni,A.O., Reed,C.D., Enomoto,T., Wenk,M.L. and Rice,J.M. (1989) Infrequent activation of K-*ras*, H-*ras* and other oncogenes in hepatocellular neoplasias initiated by methyl(acetoxymethyl)-nitrosamine, a methylating agent and promoted by phenobarbital in F344 rats. *Cancer Res.*, **49**, 1103–1109.
242. Funato,T., Yokota,J., Sakamoto,H., Kameya,T., Fukushima,S., Ito,N., Terada,M. and Sugimura,T. (1987) Activation of N-*ras* gene in a rat hepatocellular carcinoma induced by diethylnitrosamine and butylated hydroxytoluene. *Japan. J. Cancer Res.*, **78**, 689–694.
243. Buchmann,A., Bauer-Hoffman,R., Mahr,J., Drinkwater,N.R., Luz,A. and Schwarz,M. (1991) Mutational activation of the c-Ha-*ras* gene in liver tumors of different rodent strains, correlation with susceptibility to hepatocarcinogenesis. *Proc. Natl Acad. Sci. USA*, **88**, 911–915.
244. Ishikawa,F., Takaku,F., Nagao,M., Ochiai,M., Hayashi,K., Takayama,S. and Sugimura,T. (1985) Activated oncogenes in a rat hepatocellular carcinoma induced by 2-amino-3-methyl-imidazo(**4**,5-*f*)quinoline. *Japan. J. Cancer Res.*, **76**, 425–428.
245. Li,H., Lee,G.-H., Liu,J., Nomura,K., Ohtake,K. and Kitagawa,T. (1991) Low frequency of *ras* activation in 2-acetylaminofluorene- and 3′-methyl-4-(dimethylamino) azobenzene-induced rat hepatocellular carcinomas. *Cancer Lett.*, **56**, 17–24.
246. Tsuda,H., Hirohashi,S., Shimosato,Y., Ino,Y., Yoshida,T. and Terada,M. (1989) Low incidence of point mutations of c-Ki-*ras* and N-*ras* oncogenes in human hepatocellular carcinoma. *Japan. J. Cancer Res.*, **80**, 196–199.
247. Tada,M., Omata,M. and Ohto,M. (1990) Analysis of *ras* gene mutations in human hepatic malignant tumors by polymerase chain reaction and direct sequencing. *Cancer Res*., **50**, 1121–1124.
248. Ogata,N., Kamimura,T. and Asakura,H. (1991) Point mutation allelic loss and increased methylation of the c-Ha-*ras* gene in human hepatocellular carcinoma. *Hepatology*, **13**, 31–37.
249. Ochiya,T., Fujiyama,A., Fukushige,S., Hatada,I. and Matsubara,K. (1986) Molecular cloning of an oncogene from a human hepatocellular carcinoma. *Proc. Natl Acad. Sci. USA*, **83**, 4993–4997.
250. Wang,J., Chenivesee,X., Henglein,B. and Bréchot,C. (1990) Hepatitis B virus integration in a cyclin A gene in hepatocellular carcinoma. *Nature*, **343**, 555–557.
251. Tamori,A., Nishiguchi,S., Kuroki,T., Koh,N., Kobayashi,I., Yano,Y. and Otani,S. (1995) Point mutation of ornithine decarboxylase gene in human hepatocellular carcinoma. *Cancer Res.*, **55**, 3500–3503.
252. Watanabe,H., Fukuchi,K., Takagi,Y., Tomoyasu,S., Tsuruoka,N. and Gomi,K. (1995) Molecular analysis of Cip1/Waf1 (p21) gene in diverse types of human tumors. *Biochim. Biophys. Acta*, **1263**, 275–280.
253. Qin,L.-X., Tang,Z.-Y., Liu,K.-D., Ye,S.-L., He,B., Zhang,Y. and Zhou,G. (1996) Alterations of CDKN2 (p16/MTS1) exon 2 in human hepatocellular carcinoma. *Oncology Rep*., **3**, 405–408.
254. Greenblatt,M.S., Bennett,W.P., Hollstein,M. and Harris,C.C. (1994) Mutations in the *p53* tumor suppressor gene, clues to cancer etiology and molecular pathogenesis. *Cancer Res.*, **54**, 4855–4878.
255. Hsu,I.C., Metcalf,R.A., Sun,T., Welsh,J.A., Wang,N.J. and Harris,C.C. (1991). Mutational hotspot in the *p53* gene in human hepatocellular carcinoma. *Nature*, **350**, 427–428.
256. Scorsone,K.A., Zhou,Y.-Z., Butel,J.S. and Seagel,B.L. (1992) *p53* mutations cluster at codon 249 in hepatitis B virus-positive hepatocellular carcinomas from China. *Cancer Res.*, **52**, 1635–1638.
257. Fujimoto,Y., Hampton,L.L., Wirth,P.J., Wang,N.J., Xie,J.P. and Thorgeirsson,S.S. (1994) Alterations of tumor suppressor genes and allelic losses in human hepatocellular carcinoma from China. *Cancer Res.*, **54**, 281–285.
258. Bressac,B., Kew,M., Wands,J. and Ozturk,M. (1991) Selective G to T mutations of *p53* gene in hepatocellular carcinoma from southern Africa. *Nature*, **350**, 429–431.
259. Unsal,H., Yakicier,C., Marcais,C., Kew,M., Volkmann,M., Zentgraf,H., Isselbacher,K.J. and Ozturk,M. (1994) Genetic heterogeniety of hepatocellular carcinoma. *Proc. Natl Acad. Sci. USA*, **91**, 822–826.
260. Ozturk,M. and 28 collaborators (1991) *p53* mutations and hepatocellular carcinoma after aflatoxin exposure. *Lancet*, **338**, 1356–1359.
261. Hosono,S., Lee,C.-S., Chou,M.-J. Yang,C.-S. and Shih,C. (1991) Molecular analysis of the *p53* alleles in primary hepatocellular carcinomas and cell lines. *Oncogene*, **6**, 237–243.
262. Murakami,Y., Hayashi,K., Hirohashi,S. and Selliya,T. (1991) Aberrations of the tumor suppressor *p53* and retinoblastoma genes in human hepatocellular carcinoma. *Cancer Res.*, **51**, 5520–5525.
263. Oda,T., Tsuda,H., Scarpa,A., Sakamoto,M. and Hirohashi,S. (1992) *p53* gene mutation in hepatocellular carcinoma. *Cancer Res.*, **52**, 6358–6364.
264. Hollstein,M.C., Wild,C.P., Bleicher,F., Chutimataewin,S., Harris,C.C., Srivatanakul,P. and Montensano,R. (1993) *p53* mutations and aflatoxin B_1 exposure in hepatocellular carcinoma patients from Thailand. *Int. J. Cancer*, **53**, 51–55.
265. Hosono,S., Chou,M.-J., Lee,C.-S. and Shih,C. (1993) Infrequent mutation

of *p53* gene in hepatitis B virus positive primary hepatocellular carcinoma. *Oncology*, **8**, 491–496.
266. Nishida,N., Fukuda,Y., Kokuryu,H., Toguchida,J., Yandell,D.W., Ikenaga,M., Imura,H. and Ishizaki,K. (1993) Role and mutational heterogeneity of the *p53* gene in hepatocellular carcinoma. *Cancer Res.*, **53**, 368–372.
267. Nose,H., Imazeki,F., Ohto,M. and Omata,M. (1993) *p53* gene mutations and 17p allelic deletions in hepatocellular carcinoma from Japan. *Cancer*, **72**, 355–360.
268. Teramoto,T., Satonaka,K., Kitazawa,S., Fujimori,T., Hayashi,K. and Maeda,S. (1994) *p53* gene abnormalities are closely related to hepatoviral infections and occur in a late stage of hepatocarcinogenesis. *Cancer Res.*, **54**, 231–235.
269. Hayashi,H., Sugio,K., Matsumata,T., Adachi,E., Takenaka,K. and Sugimachi,K. (1995) The clinical significance of *p53* gene mutation in hepatocellular carcinomas in Japan. *Hepatology*, **22**, 1702–1707.
270. Shi,C.Y., Phang,T.W., Lin,Y., Wee,A., Li,B., Lee,H.P. and Ong,C.N. (1995) Codon 249 mutation of the *p53* gene is a rare event in hepatocellular carcinomas from ethnic Chinese in Singapore. *Brit. J. Cancer*, **72**, 146–149.
271. Kress,S., Jahn,U.-R., Buchmann,A., Bannasch,P. and Schwarz,M. (1992) *p53* mutations in human hepatocellular carcinomas from Germany. *Cancer Res.*, **52**, 3220–3223.
272. Debuire,B., Paterlini,P., Pontisso,P., Basso,B. and May,E. (1993) Analysis of the *p53* gene in hepatocellular carcinomas and hepatoblastomas. *Oncogene*, **8**, 2303–2306.
273. Kubicka,S., Trautwein,C., Schrem,H., Tillmann,H. and Manns,M. (1995) Low incidence of *p53* mutations in European hepatocellular carcinomas with heterogeneous mutation as a rare event. *J. Hepatology*, **23**, 412–419.
274. Kar,S., Jaffe,R. and Carr,B.I. (1993) Mutation at codon 249 of *p53* gene in a human hepatoblastoma. *Hepatology*, **18**, 566–569.
275. Shieh,Y.S.C., Nguyen,C., Vocal,M.V. and Chu,H.-W. (1993) Tumor-suppressor *p53* gene in hepatitis C and B virus-associated hepatocellular carcinoma. *Int. J. Cancer*, **54**, 558–562.
276. Andersson,M., Jönsson,M., Nielsen,L.L., Vyberg,M., Visfeldt,J., Storm,H.H. and Wallin,H. (1995) Mutations in the tumor suppressor gene *p53* in human liver cancer induced by α-particles. *Cancer Epid. Biomark. Prev.*, **4**, 765–770.
277. DiBenedetti,V.M.G., Welsh,J.A., Trivers,G.E., Harpster,A., Parkinson,A.J., Lanier,A.P., McMahon,B.J. and Bennett,W.P. (1995) *p53* is not mutated in hepatocellular carcinomas in Alaskan natives. *Cancer Epid. Biomark. Prev.*, **4**, 79–82.
278. DiBenedetti,V.M.G., Welsh,J.A., Yu,M.C. and Bennett,W.P. (1996) *p53* mutations in hepatocellular carcinoma related to oral contraceptive use. *Carcinogenesis*, **17**, 145–149.
279. Bjersing,L., Andersson,C. and Lithner,F. (1996) Hepatocellular carcinoma in patients from northern Sweden with acute intermittent porphyria, morphology and mutations. *Cancer Epidem. Biomark. Prev.*, **5**, 393–397.
280. Kennedy,S.M., MacGeogh,C., Jaffe,R. and Spurr,N.K. (1994) Overexpression of the oncoprotein *p53* in primary hepatic tumors of childhood does not correlate with gene mutations. *Human Pathol*., **25**, 438–442.
281. Ng,I.D.L., Srivastava,G., Chung,L.P., Tsang,S.W.Y. and Ng,M.M.T. (1994) Overexpression and point mutation of *p53* tumor suppressor gene in hepatocellular carcinomas in Hong Kong Chinese people. *Cancer*, **74**, 30–37.
282. Livni,N., Eid,A., Ilan,Y., Rivkind,A., Rosenman,E., Blendis,L.M., Shouval,D. and Galun,E. (1995) *p53* expression in patients with cirrhosis with and without hepatocellular carcinoma. *Cancer*, **75**, 2420–2426.
283. Nakopoulou,L., Janinis,J., Giannopoulou,I., Lazaris,A.C., Koureas,A. and Zacharoulis,D. (1995) Immunohistochemical expression of *p53* protein and proliferating cell nuclear antigen in hepatocellular carcinoma. *Pathol. Res. Pract*., **191**, 1208–1213.
284. Aquilar,F., Harris,C.C., Sun,T., Hollstein,M. and Cerutti, P. (1994) Geographic variation of *p53* mutational profile in nonmalignant human liver. *Science*, **264**, 1317–1319.
285. Nishida,N., Fukada,Y., Kokuryu,H. *et al*. (1992) Accumulation of allelic loss on arms of chromosomes 13q, 16q and 17p in advanced stages of human hepatocellular carcinoma. *Int. J. Cancer*, **51**, 862–868.
286. Konishi,M., Kikuchi-Yanoshita,R., Tanaka,K. *et al*. (1993) Genetic changes in human hepatocellular carcinomas. *Japan. J. Cancer Res.*, **84**, 893–899.
287. Tanaka,S., Toh,Y., Adachi,E., Matsumata,T., Mori,R. and Sugimachi,K. (1993) Tumor progression in hepatocellular carcinoma may be mediated by *p53* mutation. *Cancer Res.*, **53**, 2884–2887.
288. Hsu,H.-C., Peng,S.-Y., Lai,P.-L., Sheu,J.-C., Chen,D.-S., Lin,L.-I., Slagle,B.L. and Butel,J.W. (1994) Allelotype and loss of heterozygosity of *p53* in primary and recurrent hepatocellular carcinomas. A study of 150 patients. *Cancer*, **73**, 42–47.
289. Kuroki,T., Fujiwara,Y., Tsuchiya,E., Nakamori,S., Imaoka,S., Kanematsu,T. and Nakamura,Y. (1995) Accumulation of genetic changes during development and progression of hepatocellular carcinoma, loss of heterozygosity on chromosome 1p occurs at an early stage of hepatocarcinogenesis. *Genes, Chrom. Cancer*, **13**, 163–167.
290. Slagle,B.L., Zhou,Y.-Z. and Butel,J.S. (1991) Hepatitis B virus integration event in human chromosome 17p near *p53* gene identifies the region of the chromosome commonly deleted in virus-positive hepatocellular carcinomas. *Cancer Res.*, **51**, 49–54.
291. Ding,S.-F., Habib,N.A., Dooley,J., Wood,C. and Habib,N.A. (1991) Loss of constitutional heterozygosity on chromosome 5q in hepatocellular carcinoma without cirrhosis. *Brit. J. Cancer*, **64**, 1083–1087.
292. Fujimori,M., Tokino,T. and Hino,O. (1991) Allelotype study of primary hepatocellular carcinoma. *Cancer Res.*, **51**, 89–93.
293. Walker,G.J., Hayward,N.K., Falvey,S. and Cooksley,W.G.E. (1991) Loss of somatic heterozygosity in hepatocellular carcinoma. *Cancer Res.*, **51**, 4367–4370.
294. Malkin,D. (1994) *p53* and the Li-Fraumeni syndrome. *Biochim. Biophys. Acta*, **1198**, 197–213.
295. Oda,H., Nakatsuru,Y., Imai,Y., Sugimura,H. and Ishikawa,T. (1995) A mutational hotspot in the *p53* gene is associated with hepatoblastomas. *Int. J. Cancer*, **60**, 786–790.
296. Zhang,X., Xu,H.-J., Murakami,Y., Sachse,R., Yashima,K., Hirohashi,S., Hu,S.-X., Benedict,W.F. and Sekiya,T. (1994) Deletions of chromosome 13q, mutations in *Retinoblastoma 1* and retinoblastoma protein in human hepatocellular carcinoma. *Cancer Res.*, **54**, 4177–4182.
297. Wang,H.P. and Rogler,C.E. (1988) Deletion in human chromosome arms 11p and 13q in primary hepatocellular carcinomas. *Cytogenet. Cell Genet*., **48**, 72–78.
298. Beutow,K.H., Murray,J.C., Israel,J.L., London,W.T., Smith,M., Kew,M., Blanquet,V., Redeker,A. and Govindarajah,S. (1989) Loss of heterozygosity suggests tumor suppressor gene responsible for primary hepatocellular carcinoma. *Proc. Natl Acad. Sci. USA*, **86**, 8852–8856.
299. Abramson,D.H., Ellsworth,R.M., Kitchin,F.D. and Tung,G. (1984) Second nonocular tumors in retinoblastoma survivors. Are they radiation-induced? *Ophthalmology*, **91**, 1351–1355.
300. Meadows,A.T., Baum,E., Fossati-Bellani,F. *et al*. (1985) Second malignant neoplasms in children, an update from the late effects study group. *J. Clin. Oncol*., **3**, 532–538.
301. Breslow,N.E., Norkool,P.A., Olshan,A., Evans,A. and D'Angio,G.J. (1988) Second malignant neoplasms in survivors of Wilms' tumors, a report of the National Wilms' Tumor Study Group. *J. Natl Cancer Inst*., **80**, 592–595.
302. Koufos,A., Hansen,M.F., Copeland,N.G., Jenkins,N.A., Lampkin,B.C. and Cavanee,W.K. (1985) Loss of heterozygosity in three embryonal tumors suggests a common pathogenic mechanism. *Nature*, **316**, 330–334.
303. Rogler,C.E., Sherman,M., Su,C.Y., Summers,J., Shows,T.B., Henderson,A. and Kew,M. (1985) Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. *Science*, **230**, 319–322.
304. Little,M.H., Thomson,D.B., Hayward,N.K. and Smith,P.J. (1988) Loss of alleles on the short arm of chromosome 11 in a hepatoblastoma from a child with Beckwith-Wiedemann syndrome. *Human Genet*., **79**, 186–189.
305. Byrne,J.A., Simms,L.A., Little,M.H., Algar,E.M. and Smith,P.J. (1993) Three non-overlapping regions of chromosome arm 11p allele loss identified in infantile tumors of adrenal and liver. *Genes. Chrom. Cancer*, **8**, 104–111.
306. Albrecht,S., von Schweinitz,D., Waha,A., Kraus,J.A., von Deimling,A. and Pietsch,T. (1994) Loss of maternal alleles on chromosome arm 11p in hepatoblastoma. *Cancer Res.*, **54**, 5041–5044.
307. Iwama,T. and Michiwa,Y. (1994) Mortality in young first-degree relatives of patients with familial adenomatous polyposis. *Cancer*, **73**, 2065–2068.
308. Oda,H., Imai,Y., Nakatsuru,Y., Hata,J. and Ishikawa,T. (1996) Somatic mutations of the *APC* gene in sporadic hepatoblastoma. *Cancer Res.*, **56**, 3320–3323.
309. Simon,D., Knowles,B.B. and Werth,A. (1991) Abnormalities of chromosome 1 and loss of heterozygosity on 1p in primary hepatomas. *Oncogene*, **6**, 765–770.
310. Yeh,S.-H., Chen,R.J., Chen,H.-L., Lai,M.-Y., Wang,C.-C. and Chen,D.-S. (1994) Frequent genetic alterations at the distal region of chromosome 1p in human hepatocellular carcinoma. *Cancer Res.*, **54**, 4188–4192.
311. Simon,D. and Carr,B.I. (1995) Integration of hepatitis B virus and alteration of the 1p36 region found in cancerous tissue of primary hepatocellular carcinoma with viral replication evidenced only in noncancerous, cirrhotic tissue. *Hepatology*, **22**, 1393–1398.

312. Pasquinelli,C., Garreau,F., Bouquelleret,L. *et al*. (1988) Rearrangement of a common cellular DNA domain on chromosome 4 in primary liver tumors. *J. Virol*., **62**, 629–632.
313. Zhang,W., Hirohashi,S., Tsuda,H., Shimosato,Y., Yokota,J., Terada,M. and Sufimura,T. (1990) Frequent loss of heterozygosity on chromosomes 16 and 4 in human hepatocellular carcinoma. *Japan. J. Cancer Res.*, **81**, 108–111.
314. Yeh,S.-W., Chen,P.-J., Lai,M.-Y. and Chen,D.-S. (1996) Allelic loss on chromosomes 4q and 16q in hepatocellular carcinoma, association with elevated α-fetoprotein production. *Gastroenterology*, **110**, 184–192.
315. Emi,M., Fujiwara,Y., Nakajima,T. *et al*. (1992) Frequent loss of heterozygosity for loci on chromosome 8p in hepatocellular carcinoma, colorectal cancer and lung cancer. *Cancer Res.*, **52**, 5368–5372.
316. Fujiwara,Y., Monden,M., Mori,T., Nakamura,Y. and Emi,M. (1993) Frequent multiplication of the long arm of chromosome 8 in hepatocellular carcinoma. *Cancer Res.*, **53**, 857–860.
317. Takahashi,K., Kudo,J., Ishibashi,H., Hirata,Y. and Niho,Y. (1993) Frequent loss of heterozygosity on chromosome 22 in hepatocellular carcinoma. *Hepatology*, **17**, 794–799.
318. Tsuda,H., Zhang,W., Shimosato,Y., Yokota,J., Terada,M., Suzimura,T., Miyamura,T. and Hirohashi,S. (1990) Allele loss on chromosome 16 associated with progression of human hepatocellular carcinoma. *Proc. Natl Acad. Sci. USA*, **87**, 6791–6794.
319. Slagle,B.L., Zhou,Y.-Z., Birchmeier,W. and Scorsone,K.A. (1993) Deletion of the E-cadherin gene in hepatitis B virus-positive Chinese hepatocellular carcinoma. *Hepatology*, **18**, 757–762.
320. Simon,D., Munoz,S.J., Maddrey,W.D. and Knowles,B.B. (1990) Chromosomal rearrangements in a primary hepatocellular carcinoma. *Cancer, Genet. Cytogenet*., **45**, 255–260.
321. Chen,H.-L., Chen,Y.-C. and Chen,D.-S. (1996) Chromosome 1p aberrations are frequent in human primary hepatocellular carcinoma. *Cancer, Genet. Cytogenet*., **86**, 102–106.
322. Hino,C., Shows,R. and Rogler,C.E. (1986) Hepatitis B virus integration site in hepatocellular carcinoma at chromosome **17**, 18 translocation. *Proc. Natl Acad. Sci. USA*, **83**, 8338–8342.
323. Tokino,T., Fukushiga,S., Nakamura,T., Nagaya,T., Murotsu,T., Shiga,K., Aoki,N. and Matsubara,K.(1987) Chromosome translocation and inverted duplication with integrated hepatitis B virus in hepatocellular carcinoma. *J. Virol*., **61**, 3848–3854.
324. Meyer,M., Wiedorn,K.H., Hofschneider,P.H., Koshy,R. and Caselman,W.H. (1992) A chromosome **17**, 7 translocation is associated with a hepatitis B virus integration site in hepatocellular carcinoma DNA. *Hepatology*, **15**, 665–671.
325. Kimura,H., Kagawa,K., Deguchi,T. *et al*. (1996) Cytogenetic analysis of hepatocellular carcinoma by in situ hybridization with a chromosome-specific DNA probe. *Cancer*, **77**, 271–277.
326. Nagai,H., Ponglikitmongkel,M., Mita,E., Omachi,Y., Yoshikawa,H., Saeki,R., Yumoto,Y., Nakanishi,T. and Matsubara,K. (1994) Aberration of genomic DNA in association with hepatocellular carcinoma detected by 2-dimensional gel analysis. *Cancer Res.*, **54**, 1545–1550.
327. Haas,O.A., Zoubek,A., Grümayer,E.R. and Gadner,H. (1986) Constituional interstitial deletion of 11p11 and a pericentric inversion of chromosome 9 in a patient with Wiedemann-Beckwith syndrome and hepatoblastoma. *Cancer, Genet. Cytogenet*., **23**, 95–104.
328. Tonk,V.S., Wilson,K.S., Timmons,C.F. and Schneider,N.R. (1994) Trisomy 2, Trisomy 20 and del(17p) as sole chromosomal abnormalities in three cases of hepatoblastoma. *Genes. Chromo. Cancer*, **11**, 199–202.
329. Matsubara,K. and Tokino,T. (1990) Integration of hepatitis B virus DNA and its implication for hepatocarcinogenesis. *Mol. Biol. Med*., **7**, 243–260.
330. Ding,S.-F., Dellhanty,J.D.A., Carillo,A., Della Serra,G., Bowles,L. and Dooley,J.S. (1993) Lack of demonstrable chromosome allele loss in hepatocellular adenoma. *Int. J. Oncol*., **2**, 977–979.
331. Ding,S.-F., Jalleh,R.P., Dooley,J., Wood,C.B. and Habib,N.A. (1991) Chromosome 17 allele loss in hepatocellular carcinoma but not in synchronous hepatocellular adenoma. *Eur. J. Surg. Oncol*., **19**, 195–197.
332. Rivkina,M.B., Cullen,J.M., Robinson,W.S. and Marion,P.L. (1994) State of the *p53* gene in hepatocellular carcinomas of ground squirrels and woodchucks with past and ongoing infections with hepadna viruses. *Cancer Res.*, **54**, 5430–5437.
333. Hulla,J.E., Chen,Z.Y. and Eaton,D.L. (1993) Aflatoxin B_1-induced rat hepatic hyperplastic nodules do not exhibit a site-specific mutation within the *p53* gene. *Cancer Res.*, **53**, 9–11.
334. Lilleberg,S.L., Cabonce,M.A., Raju,N.R., Wagner,L.M. and Kier,L.D. (1992) Alterations in the structural gene and the expression of *p53* in rat liver tumors induced by aflatoxin B_1. *Mol. Carcinogenesis*, **6**, 159–172.
335. Fujimoto,Y., Hampton,L.L., Luo,L.-D., Wirth,P.J. and Thorgeirsson,S.S. (1992) Low frequency of *p53* gene mutation in tumors induced by aflatoxin B_1 in non-human primates. *Cancer Res.*, **52**, 1044–1046.
336. Fujimoto,Y., Hampton,L.L., Snyderwine,E.G., Nagao,M., Sugimura,T., Adamson,R.H. and Thorgeirsson,S.S. (1994) *p53* gene mutation and hepatocellular carcinoma induced by 2-amino-3-methylimidazo(**4**,5-*f*)quinoline in nonhuman primates. *Japan. J. Cancer Res.*, **85**, 506–509.
337. Smith,M.L., Yeleswarapu,L., Locker,J. and Lombardi,B. (1991) Expression of *p53* mutant protein(s) in diethylnitrosamine-induced foci of enzyme-altered hepatocytes in mal Fischer-344 rats. *Carcinogenesis*, **12**, 1137–1141.
338. Wirnitzer,U., Enzmann,H., Rosenbruch,M. and Bombard,E.M. (1995) Accumulation of *p53* protein in chemically induced oval cells during early stages of rodent hepatocarcinogenesis. *Carcinogenesis*, **16**, 697–701.
339. Ho,Y.-S., Cheng,H.-T., Wang,Y.-J. and Lin,J.-K. (1995) *p53* gene mutational spectra in hepatocellular carcinomas induced by 2-acetylaminofluorene and N-nitroso-2-acetylaminofluorene in rats. *Mol. Carcinogenesis*, **13**, 182–190.
340. Smith,M.L., Yeleswarapu,L., Scalamogna,P., Locker,J. and Lombardi,B. (1993) *p53* mutations in hepatocellular carcinomas induced by a choline-devoid diet in male Fischer 344 rats. *Carcinogenesis*, **14**, 503–510.
341. Fukada,I. and Ogawa,K. (1993) Detection of *p53* gene mutations in rat hepatocellular carcinoma cell lines by denaturing gradient gel electrophoresis (DGGE). *Mol. Carcinogenesis*, **7**, 257–262.
342. Smith,M.L., Yeleswarapu,L., Lombardi,B. and Shinozuka,H. (1993) Lack of mutations in the *p53* tumor suppressor gene in hepatocellular carcinomas induced in rats by a peroxisome proliferator. *Mol. Carcinogenesis*, **7**, 89–93.
343. Goodrow,T.L., Storer,R.D., Leander,K.R., Prahalada,S.R., vanZwieten,M.J. and Bradley, M.O. (1992) Murine *p53* intron sequences 5–8 and their use in polymerase chain reaction/direct sequencing analysis of *p53* mutations in CD-1 mouse liver and lung tumors. *Mol. Carcinogenesis*, **5**, 9–15.
344. Kress,S., König,J., Schweizer,J., Löhrke,H., Bauer-Hoffmann,R. and Schwarz,M. (1992) *p53* mutations are absent from carcinogen-induced mouse liver tumors but occur in cell lines established from these tumors. *Mol. Carcinogenesis*, **6**, 148–158.4
345. Rumsby,P.C., Davies,M.J. and Evans,J.G. (1994) Screening for *p53* mutations in C3H/He mouse liver tumors derived spontaneously or induced with diethylnitrosamine or phenobarbitone. *Mol. Carcinogenesis*, **9**, 71–75.
346. Calvert,R.J., Toshiro,Y., Buzard,G.S., Diwan,B.A. and Weghorst,C.M. (1995) Lack of *p53* point mutations in chemically induced mouse hepatoblastomas, an end-stage, highly malignant hepatocellular tumor. *Cancer Lett.*, **95**, 175–180.
347. Harvey,M., Vogel,H., Morris,D., Bradley,A., Bernstein,A. and Donehower,L.A. (1995) A mutant *p53* transgene accelerates tumor development in heterozygous but not nullizygous *p53*-deficient mice. *Nature Genet*., **9**, 305–311.
348. Kemp,C.J. (1995) Hepatocarcinogenesis in *p53*-deficient mice. *Mol. Carcinogenesis*, **12**, 132–136.
349. Smith,M.L., Chandar,N. and Lombardi,B. (1993) Low frequency of retinoblastoma gene alterations in rat hepatocellular carcinomas. *Mol. Carcinogenesis*, **8**, 228–233.
350. Hu,N., Gutsmann,A., Herbert,D.C., Bradley,A., Lee,W.-H. and Lee,E.Y.-H. (1994) Heterozygous Rb-$1^{\Delta 20}$/+ mice are predisposed to tumors of the pituitary gland with nearly complete penetrance. *Oncogene*, **9**, 1021–1027.
351. Williams,B.D., Remington,L., Albert,D.M., Mukai,S., Bronson,R.T. and Jacks,T. (1994) Cooperative tumorigenic effects of germ line mutations in *Rb* and *p53*. *Nature Genet*., **7**, 480–484.
352. Davis,L.M., Caspary,W.J., Sakallah,S.A., Maronpot,R., Wiseman,R., Barrett,J.C., Elliott,R. and Hozier,J.C. (1994) Loss of heterozygosity in spontaneous and chemically induced tumors of the B6C3F1 mouse. *Carcinogenesis*, **15**, 1637–1645.
353. Miyasaka,K., Ohtake,K., Nomura,K., Kanda,H., Kominami,R., Miyashita,N. and Kitagawa,T. (1995) Frequent loss of heterozygosity on chromosome 4 in diethylnitrosamine-induced C3H/MSM mouse hepatocellular carcinomas in culture. *Mol. Carcinogenesis*, **13**, 37–43.
354. Manenti,G., DeGregorio,L., Gariboldi,M., Dragani,T. and Pierotti,M.A. (1995) Analysis of loss of heterozygosity in murine hepatocellular tumors. *Mol. Carcinogenesis*, **13**, 191–200.
355. Held,W.A., Pazik,J., O'Brien,J.G., Kerns,K., Gobey,M., Meis,R., Kenny,L. and Rustum,Y. (1994) Genetic analysis of liver tumorigenesis in SV40 T antigen transgenic mice implies a role for imprinted genes. *Cancer Res.*, **54**, 6489–6495.
356. Ohsumi,T., Okazaki,Y., Okuizumi,H. *et al*. (1995) Loss of heterozygosity

in mouse hepatoma detected by systematic genome-wide scanning using RLGS genetic map. *Biochem. Biophys. Res. Commun*., **212**, 632–639.
357. Sargent,L.M., Sanderson,N.O. and Thorgeirsson,S.S. (1996) Ploidy and karyotypic alterations associated with early events in the development of hepatocarcinogenesis in transgenic mice harboring c-*myc* and transforming growth factor α transgenes. *Cancer Res.*, **56**, 2137–2142.
358. Herens,C., Alvarez Gonzalez,M.L. and Barbason,H. (1992) Cytogenetic changes in hepatocarcinomas from rats treated with chronic exposure to diethylnitrosamine. *Cytogenet. Cell Genet*., **60**, 45–52.
359. Sargent,L.M., Sattler,G.L., Roloff,B., Xu,Y.-H., Sattler,C.L., Meisner,L and Pitot,H.C. (1992) Ploidy and specific karyotypic changes during promotion with phenobarbital, 2,5,2′,5′-tetrachlorobiphenyl and/or 3,4,3′,4’-tetrachlorobiphenyl in rat liver. *Cancer Res.*, **52**, 955–962.
360. Steadman,J.S., Lee,L.W., Smith,G.J. and Grisham,J.W. (1994) DNA contents and chromosomes of transformed rat liver epithelial cells and of cells from their derived tumors. *Carcinogenesis*, **15**, 963–969.
361. Levan,G., Szpirer,J., Szpirer,C., Klinga,K., Hanson,C. and Islam,M.Q. (1991) The gene map of the Norway rat (*Rattus norvegicus*) and comparative mapping with mouse and man. *Genomics*, **10**, 699–718.
362. Coleman,W.B., McCullough,K.S., Esch,G.L., Civalier,C.J., Livanos,E., Weissman,B.E., Grisham,J.W. and Smith,G.J. (1995) Suppression of the tumorigenic phenotype of a rat liver epithelial tumor cell by the p11.2-p12 region of human chromosome 11. *Mol. Carcinogenesis*, **13**, 220–232.
363. Coleman,W.B., Presnell,S.C., Borchert,K.M., Esch,G.L., McCullough, K.D., Weissman,B.E., Smith,G.J. and Grisham,J.W. (1996) Induction of rat WT-1 expression correlates with suppression of tumorigenicity in rat liver epithelial cell lines by human chromosome 11p11.2-p12. *Cancer Res.*, submitted.
364. Okuda,K., Ohtsuki,T., Obata,H., Tomimatsu,M., Okazaki,N., Hasegawa,H., Nakajima,Y. and Onishi,K. (1985) Natural history of hepatocellular carcinoma and prognosis in relation to treatment study of 850 patients. *Cancer*, **56**, 918–928.
365. Colombo,M., DeFranchis,R., DelNinno,E. *et al*. (1991) Hepatocellular carcinoma in Italian patients with cirrhosis. *N. Engl. J. Med*, **325**, 675–680.
366. Oka,H., Kurioka,N., Kim,K., Kanno,T., Kuroki,T., Mizoguchi,Y. and Kobayashi,K. (1990) Prospective study of early detection of hepatocellular carcinoma in patients with cirrhosis. *Hepatology*, **12**, 680–687.
367. Kato,Y., Nakata,K., Omagari,K. *et al*. (1994) Risk of hepatocellular carcinoma in patients with cirrhosis in Japan. Analysis of infectious hepatitis viruses. *Cancer*, **74**, 2224–2228.
368. Shiratori,Y., Shiina, S., Imamura, M. *et al*. (1995) Characteristic differences of hepatocellular carcinoma between hepatitis B- and C-viral infection in Japan. *Hepatology*, **22**, 1027–1033.
369. McMahon,B.J., Alberts,S.R., Wainwright,R.B., Bulkow,L. and Lanier,A.P. (1990) Hepatitis B-related sequelae. Prospective study in 1400 hepatitis B surface-antigen positive Alaskan native carriers. *Arch. Intern. Med*., **150**, 1051–1054.
370. McMahon,B.J., Alward,W.L.M., Hall,D.B., Heyward,W.L., Bender,T.R., Francis,D.P. and Maynard,J.E. (1985) Acute hepatitis B virus infection, relation of age to the clinical expression of disease and subsequent development of the carrier state. *J. Infect. Dis*., **151**, 599–603.
371. Okuda,K. Nakashima, T.,Kojiro, M.,Kondo,Y. and Wada,K. (1989) Hepatocellular carcinoma without cirrhosis in Japanese patients. *Gastroenterology*, **97**, 140–146.
372. Kew,M.C. (1989) Hepatocellular carcinoma with and without cirrhosis. A comparison in Southern African Blacks. *Gastroenterology*, **97**, 136–139.
373. Kiyosawa,K., Sodeyama,T., Tanaka,E. *et al*. (1990) Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma, analysis by detection of hepatitis C virus. *Hepatology*, **12**, 671–675.
374. Okazaki,N., Yoshino,M., Yoshida,T. *et al*. (1989) Evaluation of the prognosis for small hepatocellular carcinoma based on tumor volume doubling time. *Cancer*, **63**, 2207–2210.
375. Klatskin,G. (1977) Hepatic tumors, possible relationship to use of oral contraceptives. *Gastroenterology*, **73**, 386–394.
376. Palmer,J.R., Rosenberg,L., Kaufman,D.W., Warshauer,M.E., Stolley,P. and Shapiro,S. (1989) Oral contraceptive use and liver cancer. *Am. J. Epidemiol*., **130**, 878–882.
377. Tavani,A., Negri,E., Parazzini,F., Franceschi,S. and LaVecchia,C. (1993) Female hormone utilization and the risk of hepatocellular cancer. *Brit. J. Cancer*, **67**, 635–637.
378. Sweeney,E.C. and Evans,D.J. (1976) Hepatic lesions in patients treated with synthetic anabolic steroids. *J. Clin. Pathol*., **29**, 626–633.
379. Boyd,P.R. and Mark,G.J. (1977) Multiple hepatic adenomas and a hepatocellular carcinoma in a man on oral methyl testosterone for eleven years. *Cancer*, **40**, 1765–1770.
380. Hsing,A.W., Hoover,R.N., McLaughlin,J.K., Co-Chien,H.T., Wacholder,S., Blot,W.J. and Fraumeni,J.F. (1992) Oral contraceptives and primary liver cancer among young women. *Cancer Causes Cont*., **3**, 43–48.
381. Sass,B., Rabstein,L.S., Madison,R., Nims,R.M., Peters,R.L. and Kelloff,G.J. (1975) Incidence of spontaneous neoplasms in F344 rats throughout the natural life-span. *J. Natl Cancer Inst*., **54**, 1449–1456.
382. Goodman,D.G., Ward,J.M., Squire,R.A., Chu,K.C. and Linhart,M.S. (1979) Neoplastic and non-neoplastic lesions in aging F344 rats. *Toxicol. Appl. Pharmacol*., **48**, 237–248.
383. Rice,J.M. (1969) Transplacental carcinogenesis in mice by 1-ethyl-1-nitrosourea. *Ann. N.Y. Acad. Sci*., **163**, 813–826.
384. Vesselinovitch,S.D. and Mihailovich,N. (1983) Kinetics of diethylnitrosamine hepatocarcinogenesis in the infant mouse. *Cancer Res.*, **43**, 4253–4259.
385. Fearon,E.R. and Vogelstein,B. (1990) A model for colorectal tumorigenesis. *Cell*, **61**, 759–767.
386. Moertel,C.G. (1966) Multiple primary malignant neoplasms, introduction and presentation of the data. Recent Results *Cancer Res.*, **7**, 1–21.
387. Hajdu,S.I. and Hajdu,E.D. (1968) Multiple primary malignant tumors. *J. Am. Geriat. Soc*., **16**, 16–26.
388. Nzeako,U.C., Goodman,Z.D. and Ishak,K.G. (1994) Association of hepatocellular carcinoma in North American patients with extrahepatic primary malignancies. *Cancer*, **74**, 2765–2771.
389. Lynch,H.T. and Lynch,P.M. (1979) The cancer family syndrome, a pragmatic basis for syndrome identification. *Dis. Colon Rectum*, **22**, 106–110.
390. Stein,W.D. (1991) Analysis of cancer incidence data on the basis of multistage and clonal growth models. *Adv. Cancer Res.*, **56**, 161–213.
391. DiPaolo,J.A. (1983) Relative difficulties in transforming human and animal cells *in vitro. J. Natl Cancer Inst*., **70**, 3–8.
392. Harley,C.B. and Villeponteau,B. (1995) Telomeres and telomerase in aging and cancer. *Current Opin. Genet. Dev.*, **5**, 249–255.
393. Tahara,H., Nakanishi,T., Kitamoto,M., Nakashio,R., Shay,J.W., Tahara,E., Kajiyama,G. and Ide,T. (1995) Telomerase activity in human liver tissue, comparison between chronic liver disease and hepatocellular carcinomas. *Cancer Res.*, **55**, 2734–2736.
394. Ohashi,K., Tsutsumi,M., Kobitsu,K. *et al*. (1996) Shortened telomere length in hepatocellular carcinomas and corresponding background liver tissues in patients infected with hepatitis virus. *Japan. J. Cancer Res.*, **87**, 419–422.
395. Prowse,K.R. and Greider,C.W. (1995) Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc. Natl Acad. Sci. USA*, **92**, 4818–4822.
396. Chadeneau,C., Siegel,P., Harley,C.B., Muller,W.J. and Bacchetti,S. (1995) Telomerase activity in normal and malignant murine tissues. *Oncogene*, **11**, 893–898.
397. Squire,J. and Weksberg,R. (1996) Genomic imprinting in tumours. *Semin. Cancer Biol*., **7**, 41–47.
398. Davies,S.M. (1994) Developmental regulation of genomic imprinting of the IGF2 gene in human liver. *Cancer Res.*, **54**, 2560–2562.
399. Kalscheuer,V.M., Mariman,E.C., Schepens,M.T., Rehden,H. and Ropers,H.-H. (1993) The insulin-like growth factor gene is imprinted in the mouse but not in humans. *Nature Genet*., **5**, 74–78.
400. Guengerich,F.P. (1992) Metabolic activation of carcinogens. *Pharmac. Therap*., **54**, 17–61.
401. Lin,J.H. (1995) Species similarities and differences in pharmacokinetics. *Drug Metab. Disp*., **23**, 1008–1021.
402. Eberhart,D.C., Gemczik,B., Halvorson,M.R. and Parkinson,A. (1991) Species differences in the toxicity and cytochrome IIIA-dependent metabolism of digitoxin. *Mol. Pharmacol*., **40**, 859–867.
403. Pearce,R., Greenway,D. and Parkinson,A. (1992) Species differences and interindividual variation in liver microsomal cytochrome P450 2-A enzymes, effects on coumarin, dicumarol and testosterone oxidation. *Arch. Biochem. Biophys*., **296**, 211–225.
404. Fernandez-Salguero,P. and Gonzalez,F.J. (1995) The CYP2A gene subfamily, species differences, regulation, catalytic activities and role in chemical carcinogenesis. *Pharmacogenetics*, **5**, S123–S128.
405. Lin,D.-X., Lang,N.P. and Kadlubar,F.F. (1995) Species differences in the biotransformation of the food-borne carcinogen 2-amino-1-methyl-6-phenylimidazo(**4**,5–6)pyridine by hepatic microsomes and cytosols from humans, rats and mice. *Drug Metab. Disp*., **23**, 518–524.
406. Olsen,J. and Overvad,K. (1993) The concept of multifactorial etiology of cancer. *Pharm. Toxicol*., **72**, S33-S38.
407. Guebel,A.P., Pauwels,S., Buchet,J.P., Dumont,E. and Dine,C. (1987) Increased CYT P-450 dependent function in healthy HBsAg carriers. *Pharmaceut. Therap*., **33**, 193–196.
408. DeFlora,S., Hiltanen,E., Bartsch,H., Camourano,A., Izzotti,A., Bagnaoco,M. and Millman,I. (1989) Enhanced metabolic activation of

chemical hepatocarcinogen in woodchucks infected with hepatitis B virus. *Carcinogenesis*, **10**, 1099–1106.
409. Wild,C.P., Shrestha,S.M., Anwar,W.A. and Montesano,R. (1992) Field studies on aflatoxin exposure, metabolism and induction of genetic alterations in relation to HBV infection and hepatocellular carcinoma in The Gambia and Thailand. *Toxicol. Lett.*, **64**/**65**, 455–461.
410. Kekule,A.S., Lauer,V., Weis,L., Luber,B. and Hofschneider,P.H. (1993) Hepatitis B virus transactivator HBx uses tumour promoter signalling pathway. *Nature*, **361**, 742–745.
411. Takada,S., Kido,H., Fukutomi,A., Mori,T. and Koike,K. (1994) Interaction of hepatitis B virus X protein with a serine protease, tryptase TL**2**, as an inhibitor. *Oncogene*, **9**, 341–348.
412. Wang,X.W., Forrester,K., Yeh,H., Feitelson,M.A., Gu,J.R. and Harris,C.C. (1994) Hepatitis B virus X protein inhibits *p53* sequence-specific DNA binding, transcriptional activity and association with transcription factor ERCC3. *Proc. Natl Acad. Sci. USA*, **91**, 2230–2234.
413. Huff,J. (1993) Issues and controversies surrounding qualitative strategies for identifying and forecasting cancer causing agents in the human environment. *Pharmac. Ther.*, **72**, S12-S27.
414. Moen,C.J.A., Groot,P.C., Hart,A.A.M., Snoek,M. and Demant,P. (1996) Fine mapping of colon tumor susceptibility (Scc) genes in the mouse, different from genes known to be somatically mutated in colon carcinoma. *Proc. Natl Acad. Sci. USA*, **93**, 1082–1086.
415. Su,L.-K., Kinzler,K.W., Vogelstein,B., Preisinger,A.C., Moser,A.R., Luongo,C., Gould,K.A. and Dove,W.F. (1992) Multiple intestinal neoplasia caused by mutation in the murine homolog of the APC gene. *Science*, **256**, 322–324.
416. Oshima,M., Oshima,H., Kitigawa,K., Kobayashi,M., Itakura,C. and Taketo,M. (1995) Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene. *Proc. Natl Acad. Sci. USA*, **92**, 4482–4486.
417. Reitmair,A.H., Cai,J.-C., Bjerknes,M. *et al.* (1996) MSH2 deficiency contributes to accelerated APC-mediated intestinal tumorigenesis. *Cancer Res.*, **56**, 2922–2926.
418. Nandi,S., Guzman,R.C. and Yang,J. (1995) Hormones and mammary carcinogenesis in mice, rats and humans, a unifying hypothesis. *Proc. Natl Acad. Sci. USA*, **92**, 3650–3657.

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