Role of Polychlorinated Biphenyl Exposure in the Progression of Neoplasia

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Progression is the conversion of initiated and promoted cells into cancer cells. This stage of neoplasia is characterized by an increased growth rate, metastasis, aneuploidy and evolving karyotypic instability. The stage of progression has been examined in experimental models of skin and liver cancer. Early experiments of multistage epidermal carcinogenesis demonstrated that neoplasms occur at a high frequency when complete carcinogens are applied chronically (Shubik et al., 1950). In the skin model, it was demonstrated that chronic application of an initiator to mouse skin shortened the latency to neoplasia (Roe et al., 1972; Hennings, et al., 1985). The stage of progression in liver cancer has been examined by an initiation-promotion-initiation protocol (IPI) that was first suggested by Potter (1981) and later demonstrated experimentally by Scherer (1984). In the rat liver model, the transition from preneoplastic to tumor is characterized by the appearance of foci in foci, increased chromosome damage (Pitot et al., 1989, Pitot, et al., 1991; Dragan et al., 1993; Sargent et al., 1996), and increased incidence of carcinomas (Scherer, et al., 1984; Reddy, et al., 1982). While early preneoplastic foci are karyotypically normal and do not express increased levels of oncogene products, the progression stage is characterized by increased aneuploidy and genetic instability (Scherer et al., 1984; Reddy et al., 1982; Sargent et al., 1991; Van Goethem et al., 1995). There is evidence from the mouse liver tumor model that chromosome breakage during this stage of carcinogenesis may not be random but occurs in regions of the chromosome that have linkage groups that confer tumor susceptibility (Sargent et al., 1997, 1999). These break points are later observed as deletions, amplifications, and translocations (Sargent et al., 1999). Activation of oncogenes and inactivation of suppressor genes frequently occur in regions of chromosomal break points (Hecht, et al., 1988; Glover et al., 1988; Benedict, 1987). Progression thus involves karyotypic instability, aneuploidy, gene amplification and deletion.

Compounds that induce cytogenetic damage, spindle disruption and therefore aneuploidy, are potential progressor agents. Chemicals that bind to DNA will dramatically increase chromosome damage. Polychlorinated biphenyl congeners that have a both a meta and para site available for oxidation can be metabolized through an epoxide intermediate. The epoxide intermediate, more toxic and more chromosome damaging than the parent compound (Stadnicki, 1979), has been shown to bind to DNA (McLean et al., 1996; Wyndham et al., 1976), and to be mutagenic (Forgue et al., 1979; Silberhorn et al., 1990; Preston et al., 1984). The metabolism of 2,5,2',5'-tetrachlorobiphenyl congener to an aerine oxide is favored by methylcholanthrene induction while the detoxification pathway of direct meta hydroxylation is increased by phenobarbital induction (Clevenger et al., 1989; Forgue et al., 1979; Forgue and Allen, 1982; Ishida et al., 1991; Wyndham, et al., 1978). The 3,4 oxide of 2,5,2',5'-TCB rearranges to 4-hydroxy and 3-hydroxy in a 4:1 ratio. The dihydroxy metabolite of the lower chlorinated biphenyl congeners, either by rearrangement of an epoxide or two separate hydroxylations, can be further oxidized by peroxidases and/or prostaglandin synthetase H to quinone metabolites, which can also form adducts with DNA and protein (Amaro et al., 1996; Lin et al., 1999; Oakley et al., 1992; Oakley et al., 1996; McLean et al., 1996; McLean, et al., 2000). The exposure to the planar congeners induces both the prostaglandin synthetase 2 and lipoxygenase enzymes (Lawrence et al., 1998). The oxidative damage and free radical generation is increased by the generation of superoxide dismutase (McLean et al., 2000).

Polychlorinated biphenyls cause a cascade of events primarily in the liver and immune cells, including thymic atrophy, decreased spleen weights (Silkworth et al., 1982; Harper et al., 1993), reduction of circulating lymphocytes of both the bursae and thymic cell populations, hepatomegaly and subcapsular and midzonal hepatic necrosis (Greenlee and Irons, 1981; Smialowicz et al., 1989; Durham et al., 1989; Harper, et al., 1993; Safe, 1993, Davis and Safe, 1990; Safe, 1989; Safe, 1993; Denomme et al., 1986). Polychlorinated biphenyls are potent promoters of preneoplastic foci (Oesterle, and Deml, 1981; Preston, et al., 1981). The planar congeners bind to the Ah receptor and induce cytochrome P-450 IAI and IA2 (Safe et al., 1985; Safe, 1994; McKinney et al., 1985). The nonplanar congeners are less toxic, have a low affinity for the Ah receptor, and induce P450 2B1 and 2B2. The nonplanar congeners cause hepatic enlargement and are weak promoters of preneoplastic foci in rodent liver (Oesterle and Deml, 1981; Preston et al., 1985; Davis and Safe, 1989); however, they do not cause thymic atrophy or reduction in immune function.

POLYCHLORINATED BIPHENYL CONGENERS AS CHROMOSOME DAMAGING AGENTS:

Cytogenetic studies with commercial PCB mixtures have demonstrated mixed results, many of which could be explained by the protocol chosen for these studies. Because gene amplification and deletion can result from chromosome aberrations, these studies are important to the understanding of the role of PCBs in progression. High immunosuppressive doses of Aroclor 1254 in vivo failed to yield detectable chromosome damage in direct preparations of rat bone marrow 24 hours following exposure (Green et al., 1975). Gartoff (1977) did not see chromosome damage in rat bone marrow cells 48 hours after treatment in vivo with TCDD, but did see damage after 7 days

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when bone marrow cells were allowed to divide in culture. Furthermore, when cells were not allowed to turn over in culture and were prepared directly after exposure, no chromosome damage was observed, probably because DNA synthesis is needed for chemicals to produce visible chromosome damage. Ring dove embryos from parents exposed to 10 ppm Aroclor 1254 exhibited statistically significant chromosomal aberrations (Peakall et al., 1972).

In other in vitro studies Hoopingarnar (1972) did not find chromosome damage in 3 day lymphocyte cultures exposed to Aroclor 1254 during the first 24 or first 8 hours of culture. Lymphocytes do not have the capability to metabolize PCBs until after 24 hours in culture when blast formation occurs (Gurtoo et al., 1978). In addition, Hoopingarner examined only 50 cells from one individual. Using the sample size equation of Friedman et al. (1984), Hoopingarner could only have detected a 75% level of damage with chemicals that are cytotoxic, such as PCBs (Stadnicki et al., 1979; Ohnishi and Arakawa, 1977; McKinney and Chae, 1985). Stadnicki (1979) reported that metabolites of the nonplanar congener 2,5,2',5'-tetrachlorobiphenyl would induce chromosome damage and mitotic delay in V-79 or Hela cells. While the parent compound did not cause elevated chromosome damage, the epoxide intermediate of 2,5,2',5'-Tetrachlorobiphenyl caused significant breakage.

CHROMOSOME DAMAGE WITH COMBINATIONS OF POLYCHLORINATED BIPHENYLS

Increased chromosome damage has been reported following in vitro and in vivo exposure to combinations of PCB congeners. Polychlorinated biphenyls occur as mixtures of planar and nonplanar congeners. The planar 3,3',4,4'- and the nonplanar 2,2',5,5'-tetrachlorobiphenyl are found in the Aroclor mixtures 1254, 1248 and 1242. The ratio of these two congeners in commercial mixtures was used to design exposure to the two target organs of PCB toxicity—lymphocytes and hepatocytes—in vitro and in vivo. The mutagenic properties of PCBs in human lymphocyte cultures were examined for chromosome breakage, rearrangements, sister chromatid exchange and mitotic delay in vitro and in vivo. Lymphocytes were exposed acutely in vitro to the nonplanar 2,4,5,2',4',5'-hexachlorobiphenyl, the nonplanar 2,5,2',5'-tetrachlorobiphenyl, the planar 3,4,3',4'-tetrachlorobiphenyl, or to a combination of the planar and the nonplanar congeners. The planar congener caused a dose-related chromosome breakage and mitotic delay in human lymphocytes exposed in vitro to $0.1\text{-}10^{-4}$ μg/ml. By contrast, 2,5,2',5' did not cause chromosome damage in comparable tests at doses as high as 1 μg/ml. When 3,4,3',4', at a concentration lower than that which causes chromosome damage, was combined with non-damaging doses of 2,5,2',5', the mitotic delay and chromosomal damage observed was far in excess of what one would expect from higher doses of 3,4,3',4' alone. The combination of 2,4,5,2',4',5'-hexachlorobiphenyl, however, did not cause increased chromosome aberrations. The 2,4,5,2',4',5' congener is only slowly metabolized and does not have the meta and para positions available for activation to an epoxide intermediate. The synergy of the 2,5,2',5' may have been due to metabolism though an aerine oxide intermediate and/or due to the activation of hydroxylated metabolites of 2,5,2',5' to quinones and semi quinones. The slow metabolism of the 2,4,5,2',4',5' may have prevented the activation though these nucleophiles.

The nonplanar 2,5,2',5'-tetrachlorobiphenyl and planar 3,4,3',4'-tetrachlorobiphenyl induced greater than additive toxicity in lymphocytes and hepatocytes in vivo using doses based on the in vitro examination of lymphocytes. After one year of treatment with 0.1 μg/ml 3,4,3',4'-tetrachlorobiphenyl and 10 mg/kg 2,5,2',5'-tetrachlorobiphenyl, female Sprague-Dawley rats had enlarged spleens, reduced number of circulating antibody producing cells (B-cells) and the appearance of an abnormal population of CD-4 lymphocytes in the peripheral blood. The abnormal CD-4 cells had lower levels of CD-4 protein on the membrane and a smooth surface, and they were smaller than the CD-4 cells isolated from age matched diet controls (Sargent et al., 1991). The cytogenetic preparations from the bone marrow of these rats indicated that 4.0% of the cells were aneuploid. This is approximately the size of the abnormal CD-4 population in the circulating blood. In addition, the bone marrow cells demonstrated an elevated rate of chromosome damage (Meisner et al., 1992).

The livers of the in vivo study of Sargent et al. (1992) also demonstrated a greater than additive effect of the combination of 2,5,2',5' and 3,4,3',4'-tetrachlorobiphenyl. Female Sprague-Dawley rats were given a 10 mg/kg dose of diethylnitrosamine followed by 10 mg/kg 2,5,2',5'-tetrachlorobiphenyl, 100 mg/kg 2,5,2',5'-tetrachlorobiphenyl, 0.1 mg/kg 3,4,3',4'-tetrachlorobiphenyl or phenobarbital. The low dose of 3,4,3',4' produced a moderate increase in the number of preneoplastic foci. The 10 mg/kg dose of 2,5,2',5'-tetrachlorobiphenyl did not cause a statistically significant increase of preneoplastic foci; however, the 100 mg/kg dose of the congener did cause an increase in preneoplastic foci. The combination of 3,4,3',4' and 2,5,2'5'-tetrachlorobiphenyl caused a synergistic increase in preneoplastic foci and neoplastic nodules with cellular atypia. The GGT+ hepatocytes isolated from the treated rats also demonstrated a greater than additive level of chromosome breakage, duplications and deletions (Sargent et al., 1992). There was a consistent duplication of chromosome number 1 and deletion of chromosomes 3 and 6. These chromosomal changes were later confirmed by a genome wide loss of heterozygosity analysis (Teeguarden et al., 2000). In addition to specific chromosome changes, the metaphase plates isolated from the livers of rats treated with both PCB congeners were elongated and had lagging chromosomes.

Aberrations of the mitotic spindle have been also been reported in vitro after PCB exposure. The congener, 2,5,2',5'-tetrachlorobiphenyl has been shown to cause spindle abnormalities in V79 Chinese hamster cells in vitro at 10^{-6} M concentrations. This dose is equivalent to the concentrations that are found in human blood. Lagging chromosomes, poor alignment at metaphase and depolymerization of the mitotic spindle were observed after 4 to 24 hours following dosing (Jensen et al., 2000). Other chlorinated biphenyls have been shown to bind covalently to cysteine residues of microtubule proteins, leading to the formation of micronuclei in V79 cells (Pfeiffer et al., 1996) and to induce significant oxidative DNA damage (Dahlhaus et al., 1993; Oakley et al., 1991; Oakley et al., 1992). Metabolism in vivo occurs rapidly. Quinone and semi quinone adducts can be detected 24 hours after exposure (Lin et al., 1999). In vitro metabolism of lower chlorinated PCB congeners to quinone metabolites occurs within 10 minutes (McLean et al., 1996). Due to the rapid metabolic rate, the binding of metabolites of 2,5,2'5'-tetrachlorobiphenyl to spindle proteins may be responsible for the

aneuploidy associated with 2,5,2',5' exposure in vitro. Additional studies in vivo have reported spindle aberrations following exposure to PCB congeners (Sargent et al., 1991).

PROMOTION BY A COMBINATION OF PCBS

In another promotion study with a combination of polybrominated biphenyls by R.K. Jensen and S.D. Sleight (1986), female Sprague-Dawley rats were given a single dose of N-nitrosodiethylamine (NDEA) 24 hours following a 70% partial hepatectomy. The protocol included promotion with 0.1ppm 3,3',4,4',5,5'-hexabromobiphenyl with 10 ppm 2,2',4,4',5,5'-hexabromobiphenyl in the diet for 140 days followed by a basal diet for up to another 310 days. The authors reported a greater than additive increase in the number of preneoplastic foci.

Haag-Gronlund et al. (1998) examined another combination of polychlorinated biphenyls in an initiation/promotion bioassay with 3,3',4,4'-5-pentachlorobiphenyl (PCB 126), the mono-ortho-substituted 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), and the diortho-substituted 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153). Female Sprague-Dawley rats were IP injected with 30 mg/kg N-nitrosodiethylamine 24 hours after a partial hepatectomy. Five weeks later, 15 weekly doses of the three PCBs were administered subcutaneously. The animals were sacrificed after 20 weeks of PCB administration. Weak antagonism was observed between PCB 3,3',4,4'-5-pentachlorobi-phenyl 126 and PCB 2,2',4,4',5,5'-hexachlorobiphenyl (153) for effects on volume fraction of foci, number of foci/cm^3, concentration of plasma retinol and liver retinoids, relative liver weight, and induction of CYP2B1/2. Weak antagonism was observed between PCB 126 and PCB 105 for effects on volume fraction of foci, number of foci/cm^3, and plasma retinol concentration. Weak antagonism was also observed with a combination of 2,3,4,3',4'-pentachlorobi-phenyl and 2,4,5,2',4',5'-hexachlorobiphenyl. In another promotion protocol, Berberian et al. (1995) observed an additive response on the promotion of altered hepatic foci by 3,3',4,4'-tetrachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl in rats initiated with diethylnitrosamine.

In an additional study by van der Plas (1999), Sprague Dawley rats were treated with 30 mg/kg DEN followed by a mixture of TCDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, 2,3,4,3',4'-pentachlorobiphenyl (126), 2,4,5,3',4'-pentachlorobiphenyl(PCB156), with and without 2,4,5,2',4',5'-hexachlorobiphenyl (153). TCDD alone was the positive control at 1 μg/kg/BW in corn oil. The PHAH mixture was 1 μg TEQ/kg BW/wk and 0.5, 1 and 2 μg TEQ/Kg/BW/wk. There was an increased retention of all congeners when PCB 153 was present. There was a slight increase in the number of altered hepatic foci. There was no potentiation of the EROD activity by PCB 153. The exposure level of dioxin-like compounds caused maximal induction of EROD activity. A higher level of exposure may have caused no increase in induction. Tharappel et al. (2000) reported an antagonistic result in the induction of altered hepatic foci in the livers of rats exposed to 2,4,5,2',4', 5',4'-hexachlorobiphenyl and 3,4,3',4'-tetrachlorobiphenyl. These results confirm the additive effect of these two PCB congeners in lymphocytes exposed in vitro.

The nonplanar congener 2,4,5,2',4',5'-hexachlorobiphenyl inhibited the 3,4,3',4'-tetrachlorobiphenyl or 3,4,3',4',5'-pentachlorobiphenyl-induced chicken embroytoxicity (Zhao et al., 1997). In immune cells, the combination of 2,4,5,2',4',5'-hexachlorobiphenyl with planar compounds inhibited the suppression of the immune response by 2,3,7,8-tetrachlorobiphenyl (TCDD) (Biegel et al., 1989; Harper et al., 1995). In addition, 2,5,2',5'-tetrachlorobiphenyl with iodine molecules in the 4 and 4' positions was not superadditive when combined with 2,3,7,8-tetrachlorobiphenyl (Biegel et al., 1989). This further illustrates the importance of metabolic activation at the meta and para positions. Although nonplanar PCB congeners have been shown to potentiate the Ah receptor response (Li et al., 1999; Bannister and Safe, 1987; Jongh et al., 1993a, 1993b), the metabolism of a PCB congener with both a meta and a para site unsubstituted may be critical in the synergistic interaction of two polychlorinated biphenyl compounds.

SPECIFIC CHROMOSOME CHANGES IN LIVER TUMORS

The karyotype of γ-glutamyltranspeptidase positive (GGT+) hepatocytes isolated from Sprague-Dawley rats exposed to a combination of 2,5,2',5'- and 3,4,3',4'-tetrachlorobiphenyl was examined after 7 months when prenoplastic foci were evident and at 12 months when neoplastic nodules with cellular atypia had developed. The karyotype of the GGT+ hepatocytes at 7 months had a consistent duplication of rat chromosome number 1 at bands q37 to 41. The hepatocytes of the 12 month exposure group had additional chromosomal changes including the deletion of the short arm of chromosome 3 and the long arm of chromosome 6 (Sargent et al., 1992). These chromosomal changes in GGT+ hepatocytes isolated from livers with neoplastic nodules with cellular atypia were also observed in another chemically induced model of rat liver carcinogenesis (Sargent et al., 1996b). Later examination of early prenoplastic foci and hepatocellular carcinomas isolated from Simian virus 40 T antigen transgenic rat livers at 3 and 6 months of age confirmed that the duplication of rat chromosome 1 was an early event in hepatocarcinogenesis. The examination of the karyotype of hepatocellular carcinomas demonstrated a higher frequency of the duplication of rat chromosome 1 and the loss of chromosomes 3, 6, and X as well as a loss of chromosome 15 in the rapidly dividing tumors as illustrated in figure 1. The numerous duplications of chromosome 1 in the transgenic rat tumors made it possible to narrow the altered band region to a single band, 1q41 (Figure 1; Sargent et al., 1997). The duplication of rat 1q41, deletions of rat 6 and 15 were later confirmed by microsatellite mapping with simple sequence length polymorphic markers (Teeguarden, 2000). In addition, nonrandom chromosome abnormalities of the homologus genetic linkage groups were also observed in the mouse model of liver neoplasia (Sargent et al., 1996c; Sargent et al., 1999; Figure 1). The alterations of the linkage group of rat 1q41 and of the homologus linkage group on mouse chromosome 7F1 are early events in both species (Figure 1). Susceptibility loci have been mapped in the mouse model of liver carcinogenesis. The regions that are altered on mouse chromosomes 7 and 12 contain tumor susceptibility loci for liver tumor development (Garibaldi et al., 1993). The loss of mouse chromosome 14, which has linkage groups contained on rat 15q, is a late event in both species (Figure 1). The tetratricopeptide repeat Tg737 gene is located within the deleted region on mouse chromosome 14 and

human 13q14. This gene has been reported to be deleted in both mouse and human liver tumors and has been suggested as a tumor suppressor gene in the liver (Isfort et al., 1997). Although the time course of genetic events is not established in humans, corresponding regions of the human chromosomes 11p12, 11p15.5, 14q32 and 13q14 are lost in human hepatocellular carcinoma as indicated in figure 1 (Wang et al., 1999; Wang et al., 1990; Zhang et al., 1994; Fujimoto et al., 1999; Kawamura et al., 1999).

The possible altered expression of genes within the duplicated region of rat chromosome 1 was investigated using transformed cell lines. Rat liver epithelial cell lines transfected with a mutated H-ras gene, were capable of growth in soft agar, and had a consistent duplication of chromosome 1q41. The insulin-like growth factor II (IGFII) gene is located within the duplicated region of rat chromosome 1. The cell lines had increased expression of the fetal 4.5, 2.0 and 1.0 kilobase transcripts of IGFII as well as a 5.6 kilobase transcript that was unique to the tumor cell lines (Sargent et al., 1996a). The results were confirmed at the protein level by immunohistochemistry and Western blot analysis. A M_r 26,000 and 15,000 form of the IGFII protein that was peri-nuclear was observed in the tumor cell lines. The expression of IGFII was not just a reactivation of the fetal expression of the protein. IGFII is also increased in mouse as well as human liver tumors (Carrani et al., 1991; Daughaday et al., 1990; Lomas et al., 1991). The IGFII protein is not only a liver cell mitogen; it also down regulates apoptosis (Widmer et al., 1985; Yang and Rogler, 1991; Christofori et al., 1994). The expression of an abnormal transcript of IGFII has been reported in mouse and human liver tumors (Schirmacher et al., 1992; Carrani et al., 1991; Daughaday et al., 1990; Lomas et al., 1991). Additionally, the region of mouse chromosome 7 syntenic with rat chromosome1 has been associated with susceptibility to mouse liver carcinogenesis (Yamada et al., 1994; Gariboldi et al., 1993). The duplication of the IGF II region is an early event in rat liver cancer and also in human liver cancer (Lomas et al., 1991).

Karyotypic analysis has made it possible to identify tumor susceptibility genes in many organs. Due to the differences in etiology and the limited number of human samples available, the role of specific carcinogen exposures and the time course of chromosome aberrations in human liver neoplasia have not been established. The mouse and rat liver models have been useful in establishing the role of chemicals in cytogenetic damage. In addition, cytogenetic alterations in liver tumors that are common in rat, mouse and human indicate genetic alterations that are critical to the development of liver neoplasia. These regions are good candidates for intensive mapping for tumor susceptibility genes in human liver cancer. The pattern of cytogenetic changes in the rat and mouse liver neoplasms are not dependent on the agent that is used to induce the tumor. Although the pattern of cytogenetic damage is not dependant on the agent that is used to induce liver tumors, chemicals that bind to DNA and induce chromosome damage and aneuploidy can accelerate the transition from preneoplastic to tumor. Polychlorinated biphenyls induce aneuploidy, chromosome damage, DNA adducts and proliferation of altered cells, and are progressor agents.

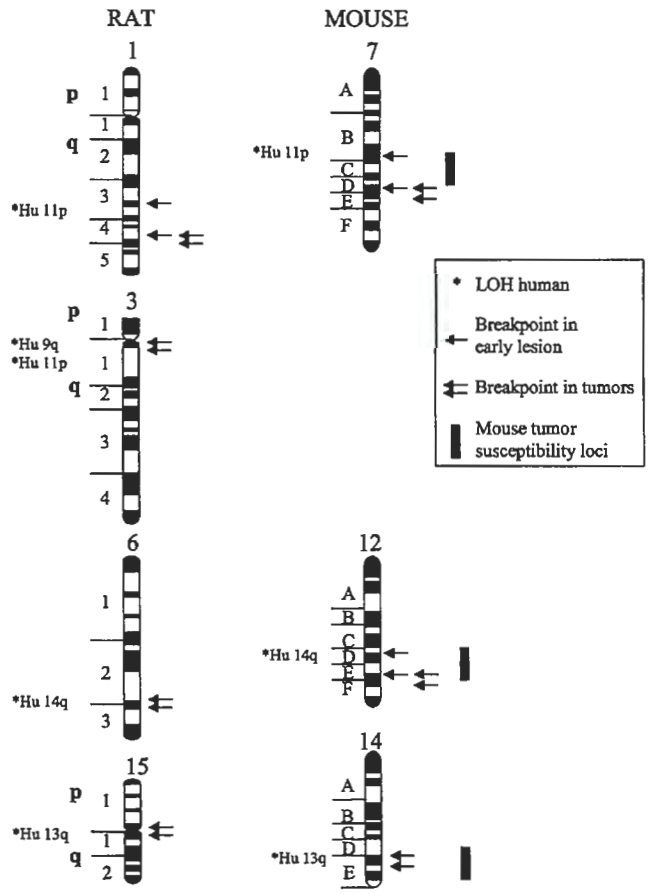

Figure 1. Ideograms of rat chromosomes 1, 3, 6 and 15 and of mouse chromosomes 7, 12 and 14. The homologus human region is indicated to the left of the ideograms as Hu. An alteration in the homologus human chromosome in liver cancer is indicated by a star. The chromosome break points that were observed in early liver lesions are indicated by single arrows, and the break points that were observed in tumors are indicated by double arrows. Tumor susceptibility loci that have been mapped in the mouse model of liver carcinogenesis are indicated by a solid bar to the right of the mouse ideogram. The altered region rat chromosome 1q37-41 is located on mouse chromosome 7F1 to the end. The homologus region of rat 6q31 to the end is located on mouse 12D1 to the end. The region on rat chromosome 15q1 to the end is located on mouse 14E1 to the end.

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