The former Division Cell Pathology (C0100) was closed after retirement of Prof. Bannasch (09/1999). Specific research topics of the Division were further pursued in the Project Group Hepatocarcinogenesis, supported by a grant and hosted by the Division Cellular and Molecular Pathology, and in the Research Group B0810 (Prof. D. Mayer).

Significance of early cellular and molecular changes for the evolution and the prevention of liver cancer

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Based on extensive previous investigations on hepatic preneoplasia and its significance for primary and secondary cancer prevention [1-4], the group prevalidated the relevance and reliability of a rat liver foci bioassay (RLFB) for the identification of cancer risks by chemicals in cooperation with the Central Unit of Biostatistics of the DKFZ and four laboratories from other institutions (Institute of Toxicology of the National Research Center for Environment and Health, Neuherberg; Product Safety - Regulations, Toxicology and Ecology, BASF AG, Ludwigshafen; Toxicology - Rodent Studies and Genetic Toxicology, Bayer AG, Wuppertal; Department of Toxicology, University of Tübingen). The four hepatocarcinogenic model compounds (N-nitrosomorpholine; 2-acetylaminofluoren, phenobarbital, and clofibrate), selected according to characteristic differences in their presumed mode of action and tested in a total of 1600 male and female rats at two dose levels each, were found to differ characteristically in their potency and dose-response relationship to induce preneoplastic foci of altered hepatocytes (FAH) when given alone or administered following initiation with diethylnitrosamine [5]. The interlaboratory variation was small for the detection of FAH by the immunohistochemical demonstration of an increased expression of the placental form of the glutathione-S-transferase (GSTP) and somewhat larger for the identification of the focal lesions in paraffin sections stained with hematoxilin-eosin. The assessment of the carcinogenic potential of the four chemicals by the different laboratories was in the same range, and the nature of their dose-response relationships did not differ essentially between laboratories. These results suggest that the RLFB prevalidated is a sensitive bioassay, providing potentially valuable information for risk assessment including the classification of carcinogenic chemicals according to their mode of action [5].

In addition to the studies aiming at primary cancer prevention by the identification and elimination of carcinogenic chemicals, the relevance of focal hepatic preneoplasia for secondary prevention of human hepatocellular carcinoma (HCC, comprising approximately 90% of the diagnosed cases of liver cancer and representing the fifth most common human cancer in the world) was investigated in 163 explanted and resected human livers, and most recently also in fine needle biopsies [6-8]. Different types of FAH (fig. 1) including glycogenic (excessively storing glycogen), basophilic, and mixed glycogenic/basophilic forms were found in 84 of 111 cirrhotic livers, demonstrating higher incidences in cases with than without HCC (fig. 2, [7]). Mixed cell populations, predominant in cirrhotic livers of the high-risk group, were more proliferative, larger and more often involved in the formation of nodular liver lesions than glycogenic cell populations. In accordance with previous findings in animal models of hepatocarcinogenesis, our findings indicate that human FAH are preneoplastic lesions, mixed cell populations being more advanced than glycogenotic foci. Small cell change within foci or nodules of altered hepatocytes is associated with an increased proliferative activity and a higher risk to

Fig. 1 Preneoplastic clear-cell focus in a liver biopsy taken from a patient with chronic hepatitis B through fine-needle aspiration. H&E. ×120, bar = 100 µm. (from [7])
and enhanced by oral administration of aflatoxin B1 [6]. Including HCC, the appearance of which is accelerated by cirrhosis with hepatocellular carcinoma (HCC) [9]. Appearance of WHV replicative intermediates and expression of antigens were limited to the substrate-1, glycogen synthase kinase β, catalytic and glycogen-binding subunit of protein phosphatase-1, protein kinase A, γ-subunit of phosphorylase kinase, glycogen phosphorylase, K-ras oncogene) in normal rat liver, and in preneoplastic and neoplastic liver cell populations using different molecular biological approaches: Northern blots, ribonuclease protection assay, quantitative real time RT-PCR, automatic DNA sequencing. In contrast to the results of previous studies by other authors, we were able to show that the inhibitor-1 of protein phosphatase-1 (PP1) is expressed in normal liver of the rat as in other species [11, 12]. In all glycogen-poor HCCs induced in rats by N-nitrosomorpholine and in a glycogen-poor rat liver cell line, inhibitor-1 mRNA was increased, while it was equal or slightly decreased in a preneoplastic glycogenotic liver cell line [12]. In addition, the investigation of the preneoplastic glycogenotic liver cell line revealed that the accumulation of glycogen results from altered expression of several genes related to the insulin and the cAMP-signaling pathways [12]. No mutations in the coding sequences of the respective genes were detected, suggesting epigenetic rather than genetic causes of the preneoplastic metabolic changes.

Fig. 2  Semiquantitative evaluation of hepatic focal lesions (relative areas occupied: + = <1%; 2+ = 1-30%; 3+ = ≥30%), large-cell change (LCC: + = low grade; 2+ = moderate grade; 3+ = high grade), intrafocal (FSCC) and diffuse (DSCC) small-cell change (LCC: + = low grade; 2+ = moderate grade; 3+ = high grade), and low-risk (C) cirrhoses without HCC, and the slightly disordered control livers (D), with groups A and C statistically compared to the counterparts in group B (* = P<0.01; ** = P<0.05; NS = not significant). MCF = mixed cell foci; GS = glycogen-storing foci; APF = amphophilic cell foci; OCF = oncocytic foci. (from [7])

nodular transformation, and, hence, should be considered a precancerous condition. Most recently, FAH were also detected in fine-needle liver aspiration biopsies, suggesting their use for monitoring HCC development in high-risk populations, such as hepatitis B virus (HBV) carriers with chronic hepatitis and/or cirrhosis.

Chronic infection of the liver with HBV and the ingestion of foodstuffs contaminated with mycotoxins, particularly aflatoxin B1, have been identified as major risk factors for the development of HCC in humans. In woodchucks, the woodchuck hepatitis virus (WHV), a DNA virus belonging to the same family as HBV (hepadnaviridae), induces hepatic alterations similar to those of human HBV-carriers, including HCC the appearance of which is accelerated and enhanced by oral administration of aflatoxin B1. Using this animal model, we investigated WHV replication as detected by in situ hybridization for WHV DNA and immunohistochemical demonstration of WHV core (WHCV Ag) and surface antigens (WHVS Ag) in preneoplastic hepatic cellular lineages [9]. Appearance of WHV replicative intermediates and expression of antigens were limited to the cytoplasm of hepatocytes and were strongly correlated (p<0.0001), both showing high levels in areas characterized by minimal structural deviations, but markedly reduced amounts in all types of preneoplastic hepatocellular focus (p<0.0001), and in hepatic adenomas (fig. 3). Most HCC were negative for WHV replicative intermediates and antigens. We conclude from these findings that WHV replication and antigen expression gradually decrease early during the preneoplastic phase. The close correlation of these changes with metabolic alterations characterizing preneoplastic hepatic cellular lineages [1-4] suggests that oncogenic effects mimicking insulin/glucagon imbalances may be responsible for the repression of hepadnaviral replication [9]. In contrast to some other authors, we were unable to detect an expression of the HBV X gene-encoded protein in a human cholangiocarcinoma and its surrounding, which had developed in an HBV-associated liver cirrhosis [10].

Extending earlier studies of our laboratory on changes in cellular signaling and metabolic patterns in hepatic preneoplasia [1-4], we jointly with two other laboratories of the DKFZ investigated the expression and structure of several genes involved in glycogen metabolism (insulin receptor substrate-1, glycogen synthase kinase β, catalytic and glycogen-binding subunit of protein phosphatase-1, protein kinase A, γ-subunit of phosphorylase kinase, glycogen phosphorylase, K-ras oncogene) in normal rat liver, and in preneoplastic and neoplastic liver cell populations using different molecular biological approaches: Northern blots, ribonuclease protection assay, quantitative real time RT-PCR, automatic DNA sequencing. In contrast to the results of previous studies by other authors, we were able to show that the inhibitor-1 of protein phosphatase-1 (PP1) is expressed in normal liver of the rat as in other species [11, 12]. In all glycogen-poor HCCs induced in rats by N-nitrosomorpholine and in a glycogen-poor rat liver cell line, inhibitor-1 mRNA was increased, while it was equal or slightly decreased in a preneoplastic glycogenotic liver cell line [12]. In addition, the investigation of the preneoplastic glycogenotic liver cell line revealed that the accumulation of glycogen results from altered expression of several genes related to the insulin and the cAMP-signaling pathways [12]. No mutations in the coding sequences of the respective genes were detected, suggesting epigenetic rather than genetic causes of the preneoplastic metabolic changes.

Publications (* = external co-author)


Fig. 3 Woodchuck hepatitis virus (WHV) DNA replication and expression of surface antigen (WHsAg) and core antigen (WHcAg) in preneoplastic (a-m) and neoplastic (n-q) liver lesions from chronic WHV carriers, as demonstrated in serial paraffin sections by *in situ* hybridization for DNA (b, f, k, o) and polyclonal antibodies to WHsAg (c, g, l, m, p) and WHcAg (d, h, q). a-d) Minimal deviation area (a, H&E) showing distinct WHV DNA replication (b) and expression of WHsAg (c) and WHcAg (d) in the majority of cells. All figures: x 200. e-h) Amphiphilic cell focus showing loss of glycogen (e, PAS-reaction), down-regulation of WHV DNA replication (f) and expression of WHsAg (g) and WHcAg (h). All figures: x 90. i-l) Glycogenotic focus showing clear or pale cells after elution of the glycogen (i, H&E) and downregulation of WHV DNA replication (k) and expression of WHsAg (l). All figures: x 80. m) Portion of amphiphilic cell focus including ground-glass hepatocytes showing preferential expression of membrane-associated WHsAg, and strong intra-cytoplasmic expression of WHcAg in some ground-glass hepatocytes. x 180. n-q) Subpopulation of hepatocellular carcinoma showing amphiphilic character of neoplastic cells (n, H&E), pronounced WHV DNA replication (o), and weak expression of WHsAg (p) and WHcAg (q). All figures: x 220. (from [9])