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Human hepatic preneoplasia: phenotypes and proliferation kinetics of foci and nodules of altered hepatocytes and their relationship to liver cell dysplasia

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Abstract Foci of altered hepatocytes (FAH) represent preneoplastic lesions, as shown in various animal models of hepatocarcinogenesis, but their significance in the human liver has not been established. The cellular composition, size distribution and proliferation kinetics of FAH in 163 explanted and resected human livers with or without hepatocellular carcinoma (HCC) and their possible association with small-cell change of hepatocytes (SCC) were therefore studied. FAH, including glycogen-storing foci (GSF), mixed cell foci (MCF) and basophilic cell foci, were found in 84 of 111 cirrhotic livers, demonstrating higher incidences in cases with (29/32) than in those without HCC (55/79). FAH were observed more frequently in HCC-free cirrhosis associated with hepatitis B or C virus or chronic alcoholic abuse (high-risk group) (37/47) than in that due to other causes (low-risk group) (12/21). MCF, predominant in cirrhotic livers of the high-risk group, were more proliferative, larger and more often involved in formation of nodules of altered hepatocytes (39.3%) than were GSF (8.5%). The results suggest that the FAH are preneoplastic lesions, MCF being more advanced than GSF. Oncocytic and amphophilic cell foci were also observed, but their significance remains to be clarified. Two types of SCC, namely diffuse and intrafocal SCC, were identified, but only intrafocal SCC was found to be related to increased proliferative activity and more frequent nodular transformation of the FAH involved, suggesting a close association with progression from FAH to HCC.

Key words Foci of altered hepatocytes · Liver cell dysplasia · Liver cirrhosis · Hepatocellular carcinoma · Proliferating cell nuclear antigen

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Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent malignant neoplasms in human beings. Chronic infection with the hepatitis B virus (HBV) or the hepatitis C virus (HCV), ingestion of food contaminated with chemical carcinogens, especially aflatoxin B_1, and alcoholic beverages are considered major risk factors for HCC development [7, 15, 18]. Molecular analyses have shown that activation of some proto-oncogenes and genes for growth factors, mutations of the *p53* gene or even dysfunction of its encoded protein may play a role in certain stages of human hepatocarcinogenesis [7, 34, 54, 62, 64]. Different lesions have been suggested to represent preneoplastic conditions, with two types of liver cell dysplasia, namely large-cell and small-cell, being reported. The large-cell change of hepatocytes (LCC) described by Anthony et al. in 1973 [3] has been regarded as a precancerous lesion by some authors [3, 14, 17], but its high prevalence in many chronic liver diseases, near-normal cytoplasmic/nuclear ratio and low proliferative activity does not lend support to this notion [22, 37, 42, 51, 63, 68]. The small-cell change of hepatocytes (SCC), also known as small-cell dysplasia, was described in cirrhosis and chronic hepatitis by Watanabe et al. [68]. Similarities in the morphological and antigenic cellular

phenotypes between SCC and well-differentiated HCC suggest that SCC in liver parenchyma is a precancerous lesion [29, 44, 63, 68, 74]. In addition, Japanese pathologists regard SCC in cirrhotic liver as an important morphological feature of "extremely well-differentiated HCC" [54]. However, the nature of SCC is still controversial [49, 74]. During the last decade, adenomatous hyperplasia in the cirrhotic liver has been described and investigated extensively [29, 37, 44]. It has been noted that adenomatous hyperplastic nodules of "ordinary" type are similar to other regenerative nodules in their biological behaviour, while those with "hepatocytic atypia" are thought to be borderline lesions [29, 37, 44, 54]. The hepatocytic atypias have never been well defined, however, and unequivocal distinction between these two types of nodular lesions and between the nodules with hepatocytic atypia and well-differentiated HCC remains problematic [29, 39].

Foci of altered hepatocytes (FAH) are found in several animal species in the early stages of hepatocarcinogenesis caused by chemicals, radiation, chronic infection with hepadnaviruses and in some transgenic models [7]. Their preneoplastic nature has been well documented [8, 10, 57, 71], with preneoplasia defined as phenotypically altered cell populations that have no neoplastic nature but are endowed with an increased risk of developing into either benign or malignant neoplasms [6]. Similar lesions have been observed fortuitously in the livers of women with long-term use of oral contraceptives and in other pathologic conditions [2, 5, 16, 30, 35, 42], particularly in genetic haemochromatosis [25]. Recently, Bannasch et al. [11] described the biochemical phenotype of FAH, including glycogen-storing clear cell foci and clear cell-predominating mixed cell foci, in more than 50% of cirrhotic livers with or without HCC. In this study, we extended the number of cases investigated to a total of 163 explanted and resected liver specimens and analysed the morphological phenotype and proliferation kinetics of various types of FAH. We paid special attention to the association of FAH with so-called liver cell dysplasia, particularly SCC, and with chronic liver diseases involving high and low risk of HCC.

Materials and methods

Tissue samples were taken from a total of 163 explanted and resected livers (Table 1). In addition to 144 explants with advanced primary liver disease, 7 donor livers and 12 livers with space-occupying lesions other than HCC were also studied for comparison. The 12 livers with other space-occupying lesions included 6 explants (3 with polycystic liver disease, 1 with an adenocarcinoma of bile duct, 1 with a malignant epithelioid haemangioendothelioma and 1 with a metastatic carcinoid) and 6 resected specimens (2 with focal nodular hyperplasia, 2 with metastatic colon adenocarcinomas, 1 with a metastatic breast adenocarcinoma and 1 with echinococcosis). The study protocol was approved by the Ethics Commission of the Medical Faculty of the University of Heidelberg. All livers were removed from the patients within 45 min, and samples were fixed in Carnoy's solution and embedded in paraffin as described previously [11]. Serial sections 3 μm in thickness were prepared.

For each tissue sample, sections were stained with haematoxylin and eosin (H&E), the periodic acid–Schiff-reaction (counterstained with orange G and iron haematoxylin, Tri-PAS) for the demonstration of glycogen, and with Azan-Mallory trichrome and Gomori. Sections of three to five tissue samples for each case were also stained immunohistochemically for the demonstration of the proliferating cell nuclear antigen (PCNA) using mouse monoclonal antibody PC10 (Dako, Carpinteria, USA) diluted 1:1600. HBV surface antigen (HBsAg) and core antigen (HBcAg) were detected using mouse monoclonal antibody 3E7 (Dako, 1:300) and a rabbit polyclonal antibody (B0586, Dako, 1:3000), respectively. The reactions were demonstrated by the alkaline phosphatase-labelled streptoavidin–biotin complex (LSAB kit, AP, Dako) and developed with new fuchsin as described previously [11]. The sections were counterstained with Mayers haematoxylin and mounted with Kaiser's glycerol gelatin (E. Merk, Darmstadt, Germany). Cytokeratin 18-immunoreactivity was demonstrated with the same procedure using mouse monoclonal antibody Ks18.04 (Progen, Heidelberg, Germany) diluted 1:150 as a positive control. For negative controls, normal rabbit immunoglobulin fraction 4 (Dako) and mouse monoclonal antibody control Clone 679.1Mc7 (Immunotech, Hamburg, Germany) substituted for primary rabbit and mouse antibodies, respectively, at the same concentrations.

At least 5 cm^2 total areas were observed microscopically for each specimen for histological diagnosis and detection of FAH and other putative preneoplastic lesions. Histological diagnosis was made by three pathologists (Q.S., P.B., and W.J.H.) independently. Infections of HBV and HCV were confirmed by serological methods, immunohistochemical detection of HBV antigens and detection of anti-HCV antibodies, respectively. HCC was diagnosed using the World Health Organization classification [40] and graded histologically for degree of differentiation from grade I to grade IV according to the criteria of Edmondson and Steiner [26]. In this study, grade I HCC was diagnosed only for the lesions composed of small cells with pronounced nuclear and architectural atypia, with reference to the suggestions made by an International Working Party [39]. An two-fold increase in cellular density compared with surrounding parenchyma, hepatic plates more than three cells thick with a disturbed arrangement, and an expansive or invasive growth pattern were used as the diagnostic indicators of HCC. HCCs of grades I and II were referred to as well differentiated, and grades III and IV HCCs as poorly differentiated.

LCC was identified following the criteria of Anthony et al [3] and classified into low (+), moderate (2+) and high (3+) grades as described by Cohen and Berson [22]. SCC was identified by the criteria of Watanabe et al [68] and divided into low (+) and high (2+) grades as described by Su et al. [63]. Briefly, + was defined as only a single or a few small lesions composed of small hepatocytes forming hepatic plates two to three cells in thickness, and presenting a 1.5- to 2-fold increase in cellular density compared with the surrounding extrafocal parenchyma; 2+ was larger lesions composed of small hepatocytes with nuclear and architectural atypia (disturbed hepatic plates three cells thick and a two-fold increase in cellular density), but without sufficient features of malignancy to allow a definite diagnosis of HCC.

FAH were identified mainly in sections stained with H&E and PAS by two pathologists (Q.S. and P.B.) separately. The corresponding lesions in the immunostained adjacent sections were located by careful observation and exact comparison with H&E- and PAS-stained sections. Only well-demarcated foci of phenotypically altered hepatocytes that were distinguishable from the surrounding parenchyma were identified as FAH, and these were classified into glycogen-storing foci (GSF), amphophilic cell foci (APF), mixed cell foci (MCF), oncocytic foci and basophilic cell foci according to the well established criteria in rodents [10, 33] and man [2]. MCF with amphophilic and basophilic hepatocytes, respectively, were defined as clear/amphophilic (MCFA) and clear/basophilic (MCFB) mixed cell foci. Advanced FAH, which were usually more than 1 mm^2 in cross-section area and compressed the surrounding parenchyma, were defined as nodules of altered hepatocytes in this study. The relative areas made up of FAH were graded as: no FAH (–); only one or a few FAH smaller than 0.16 mm^2 within an area of 1 cm^2 (<1% of the total area)

Table 1 Foci of altered hepatocytes and liver cell dysplasia in 150 explanted, and six resected livers of patients with different liver diseases, and in seven donor livers (*HCC* hepatocellular carcinoma, *FAH* foci of altered hepatocytes, *APF* amphophilic cell foci, *FSCC* intrafocal small-cell change, *DSCC* diffuse small-cell change, *LCC* large-cell change, *HBV* hepatitis B virus, *HCV* hepatitis C virus)

Specimens	Number of patients (male/female)	Number with HCC (%)	Age		Number with				
			Mean (HCC+/HCC–)	Range	FAH (%)	APF (%)	FSCC (%)	DSCC (%)	LCC (%)
Cirrhosis	111 (73/38)	32 (28.8)	44.6 (51.3/41.9)	2–69	84 (75.7)	50 (45.1)	28 (25.2)	5 (4.5)	55 (49.6)
Posthepatitic, HBV[a]	28 (24/4)	15 (53.6)	46.0 (47.7/44.0)	31–63	25 (89.3)	16 (57.1)	10 (35.7)	0	21 (75.0)
Posthepatitic, HCV	23 (16/7)	8 (34.8)	43.7 (51.6/39.4)	20–68	21 (91.3)	8 (34.8)	6 (26.1)	0	8 (34.8)
Posthepatitic, autoimmunity	3 (0/3)	0	37.3	17–54	1 (33.3)	1 (33.3)	0	2 (66.7)	1 (33.3)
Alcoholic	27 (21/6)	8 (29.6)	49.9 (56.0/47.1)	28–69	19 (70.4)	14 (51.9)	9 (33.3)	0	13 (48.2)
Cryptogenic	12 (7/5)	1 (8.3)	43.0 (65 /41.0)	4–65	7 (58.3)	7 (58.3)	3 (25.0)	0	6 (50)
Biliary	10 (1/9)	0	50.1	34–64	7 (70)	3 (30.0)	0	3 (30.0)	4 (40.0)
Other known associations	8 (4/4)	0	23.1	2–40	4 (50)	1 (12.5)	0	0	2 (25)
Primary sclerosing cholangitis[b]	9 (4/5)	0	50.8	34–61	8 (88.9)	5 (55.6)	0	4 (44.4)	5 (55.6)
Noncirrhotic livers with hepatocellular malignancies[c]	5 (2/3)	4 (80)	21.8 (27.0/1)[d]	1–39	4 (80)	3 (60)	2 (40)	0	2 (40)
Metabolic diseases, noncirrhotic[e]	4 (1/3)	0	16.8	4–22	1 (25)	0	0	0	1 (25)
Acute or subacute massive necrosis	15 (4/11)	0	28.9	5–51	0	0	0	0	3 (20.0)
Other space-occupying lesions	12 (4/8)	0	48.3	23–65	4 (33.3)	0	0	0	1 (8.3)
Donor liver	7 (2/3)[f]	0	33.4	3–54	2 (28.6)	0	0	0	0

[a] Two with coinfection of HCV
[b] Two cases with cholangiocellular carcinoma, four with cirrhosis and four with advanced fibrosis
[c] Two cases with ordinary HCC, two with HCC of fibrolamellar type and one with hepatoblastoma and HBV infection
[d] HCC/hepatoblastoma
[e] One case each with cystinosis, genetic glycogen-storing disease Ia, protoporphyria, and oxalosis
[f] Age and sex cannot be found for two donors

(+); many FAH occupying 1–30% (2+) or ≥ 30% (3+) of the total area.

Size, cell density and proliferative activity of the focal lesions were evaluated in representative sections randomly selected from 78 cases of specimens containing FAH. All intact FAH were identified, subtyped and located in the immunostained adjacent sections. Because comparative quantitative investigations of FAH revealed that expression of the data for actually measured two-dimensional areas and from three-dimensional extrapolation gave essentially similar results [38], the size of individual lesions was estimated by measuring the cross-section areas and expressed in square millimetres. The measurement was carried out with an ocular grid and a ×25 objective lens. For the evaluation of the cell density, all nucleated hepatocytes of each lesion were counted, and the data were expressed as number per square millimetre. The cell density of extrafocal parenchyma was assessed by measuring five randomly selected areas of each section containing FAH. The same numbers of selected areas were examined in sections of donor livers. Each area covered 0.8464 mm² (four grids with a ×25 objective lens).

Sections stained for PCNA were used to evaluate the proliferative activity. Only moderately to strongly labelled hepatocyte nuclei were regarded as positive. Levels of proliferation were expressed in PCNA-labelling indices (PCNA-LI) (number of positive nucleated hepatocytes/total number of nucleated hepatocytes × 100%). Eighty-six mini-FAH composed of less than 50 hepatocytes were excluded from the PCNA-LI assessment to ensure the reproducibility.

Quantitative data were expressed as median±standard error, and mean values were given to indicate the data for ages of patients. The Kruskal–Wallis rank-sum test and the two-sided Wilcoxon rank-sum test [46] were sequentially applied to analyse the data using the software package S-PLUS, Version 3.3 (MathSoft, Inc., Seattle, USA). The resulting *P* -values were adjusted using a modified Bonferroni procedure [36]. Comparison of ordinal data was carried out with the Cochran-Armitage test [4, 21] using the software package StatXact-Turbo, Version 2.0 (Cytel Software Corp., Cambridge, Mass.). The nonparametric curve-fitting method *loess* (local regression) [20] was used to describe the relationship beween PCNA-LI and cell density. Group differences were analysed with the Chi-square test. $P < 0.05$ was always regarded as indicating a significant difference.

Results

FAH, mainly GSF and MCF, were detected in 76% (84/111) of cirrhotic livers, and in four of the five noncirrhotic livers with hepatocellular malignancies including ordinary HCC (2/2), hepatoblastoma (1/1) and HCC of fibrolamellar type (1/2). FAH, mainly small GSF, were also found in primary sclerosing cholangitis with or without cholangiocellular carcinoma. Mini-GSF were observed in 4 of the 12 livers bearing other space-occupying lesions, along with perivenular storage of fat and slight portal fibrosis in all cases. Mini-GSF were also observed in 2 of the 7 donor livers, in association with slight perivenular fibrosis in 2, and perivenular storage of fat in 1 case. The parenchymal liver tissue harbouring other space-occupying lesions and donor livers were pooled together, and regarded as a (slightly disordered) control group for calculating the incidence of FAH and evaluating their average size, because only slight morphological changes were found, and low incidences and small sizes of GSF were observed in both groups (Table 1).

As demonstrated in Table 1, most (32/36) of the HCCs in this series were observed in liver cirrhoses,

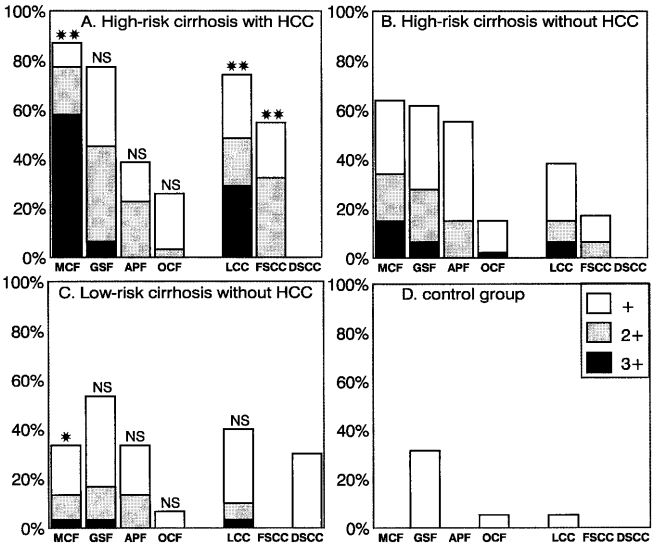
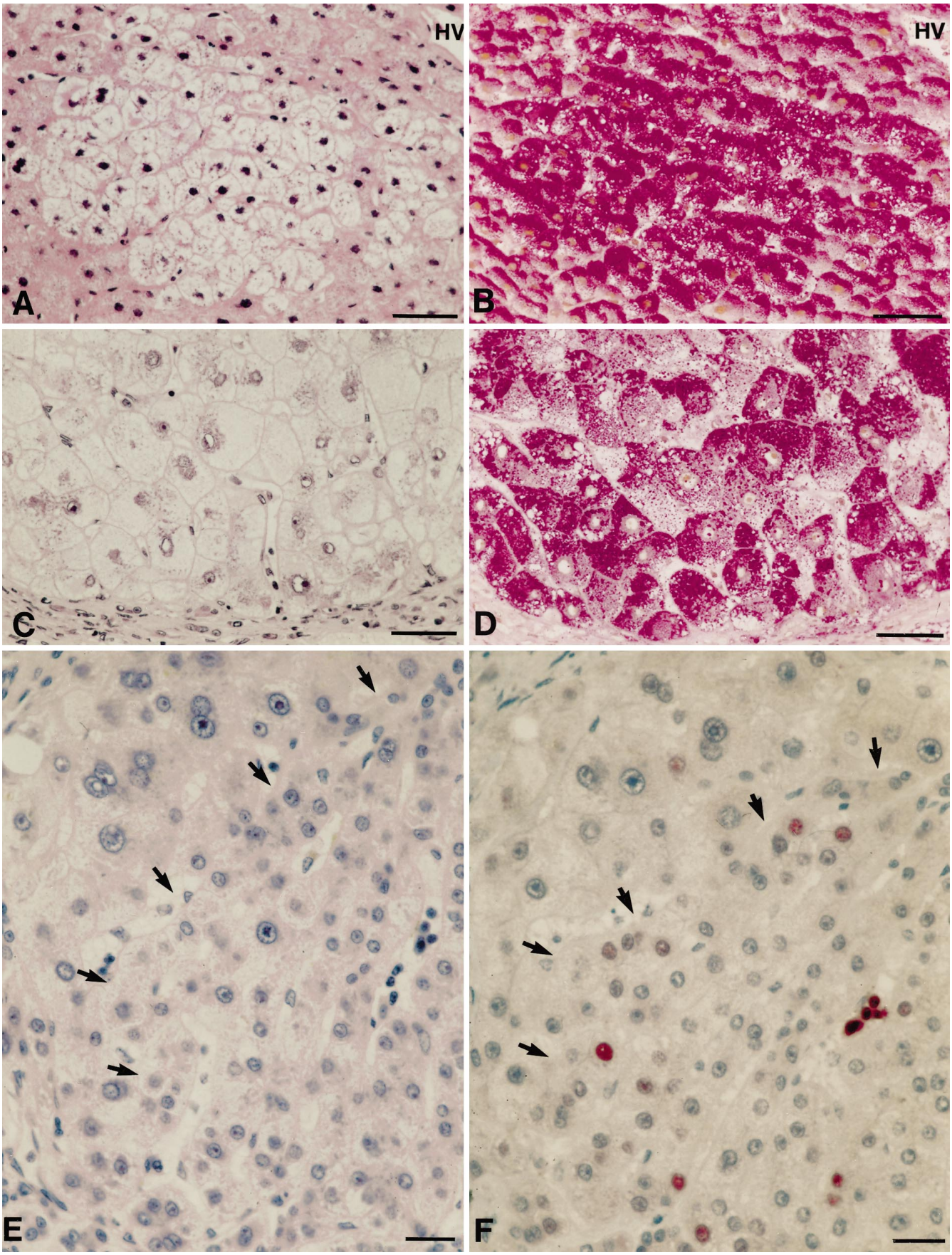


Fig. 1 A–D Semiquantitative evaluation of hepatic focal lesions (relative areas occupied: + <1%, 2+ 1–30%, 3+ ≥30% of total area), large-cell change (*LCC*: + low grade, 2+ moderate grade, 3+ high grade), intrafocal (*FSCC*) and diffuse (*DSCC*) small-cell change of hepatocytes (+ low grade, 2+ high grade) in **A**, **B** high-risk cirrhosis **A** with and **B** without hepatocellular carcinoma (*HCC*), **C** low-risk cirrhosis without HCC, and **D** in the slightly disordered control livers, with groups **A** and **C** statistically compared with the counterparts in **B** (✶✶ $P < 0.01$, ✶ $P < 0.05$, *NS* not significant). *MCF* mixed cell foci, *GSF* glycogen-storing foci, *APF* amphophilic cell foci, *OCF* oncocytic foci

mainly caused by HBV or HCV infection or by alcoholic liver disease, which are considered high-risk factors for development of HCC [7, 15, 18, 54]. They were, therefore, regarded as constituting a high-risk group for the present study. The incidence of FAH in cirrhotic livers bearing HCC (29/32) was significantly higher than in those without HCC (55/79) ($P < 0.05$). Among HCC-free cirrhoses with known causes ($n = 68$), the incidence of FAH in cirrhoses associated with high risk factors (37/47) was still higher than in primary biliary cirrhosis, and in cirrhosis due to autoimmune hepatitis and other known associations (12/21; $P < 0.05$). The latter included 3 cases of Byler's disease, 2 cases of Budd-Chiari's syndrome, 1 case of genetic haemochromatosis, 1 case of α_1-antitrypsin deficiency and 1 case of Wilson's disease. All these diseases were reported to confer a low risk for the development of HCC [25, 28, 50, 52, 53, 58, 67], so we pooled them together as the low-risk group for statis-

Fig. 2 Glycogen-storing foci (GSF) **A–D** without and **E, F** with intrafocal small-cell change (FSCC). **A, B** Perivenular GSF in an HCC-bearing liver with hepatitis B virus (HBV)-associated cirrhosis, demonstrated in serial sections with **A** H&E and **B** periodic acid Schiff–reaction counterstained with orange G and iron hematoxylin (Tri-PAS). *HV* hepatic venule. **C**, **D** Clear cell nodule in an HCC-free liver with HBV-associated cirrhosis, serial sections. **C** H&E, **D** Tri-PAS, ×120, *bar* 100 μm. **E**, **F** Increased cell proliferation in GSF (*arrows*) with less pronounced storage of glycogen (not shown) and with low-grade FSCC in a liver with cryptogenic cirrhosis, demonstrated in serial sections. **E** H&E, **F** proliferating cell nuclear antigen (PCNA) immunostaining, ×180, *bar* 50 μm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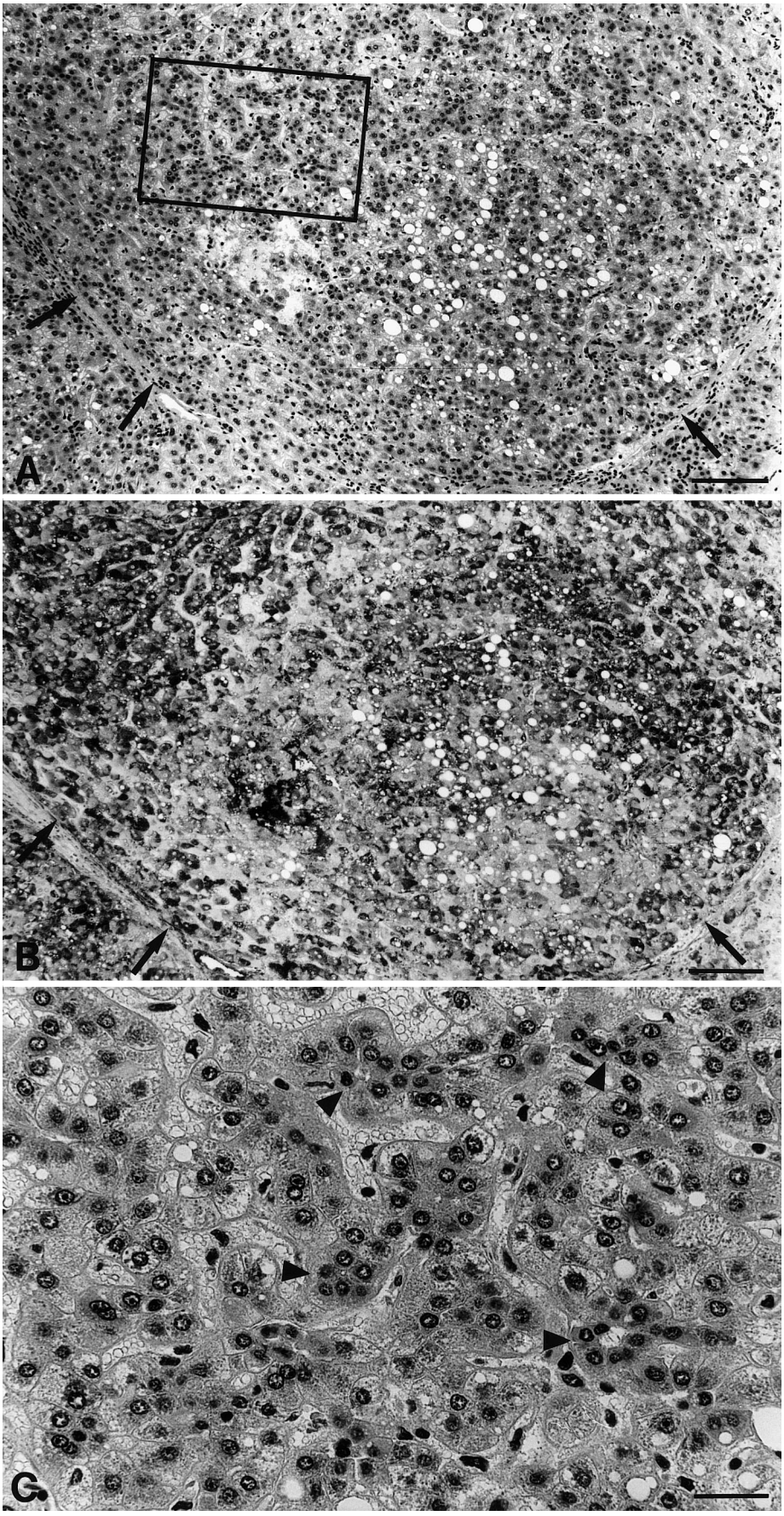


Fig. 3 **A–C** Portion of HCC-bearing cirrhotic liver with hepatitis C virus (HCV) infection. **A, B** Clear/amphophilic mixed cell nodule (*arrows*) with an area of low-grade intrafocal small-cell change (*frame*), serial sections. **A** H&E, **B** Tri-PAS, ×60, *bar* 200 μm. **C** Area within the *frame* in **A**, occupied by clusters of small hepatocytes (*arrowheads*) forming thickened hepatic plates with dilated canaliculi. ×240, *bar* 50 μm

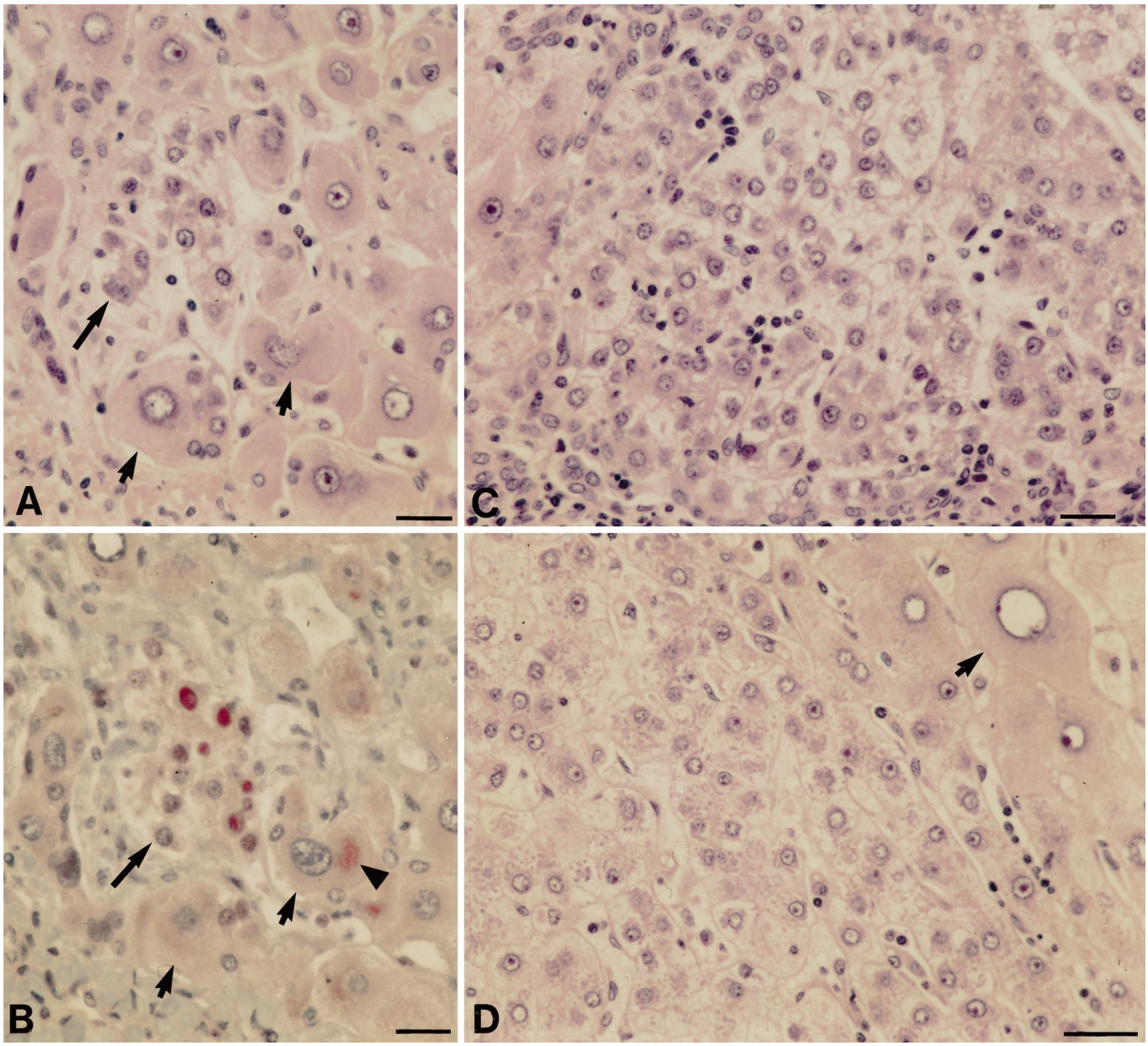
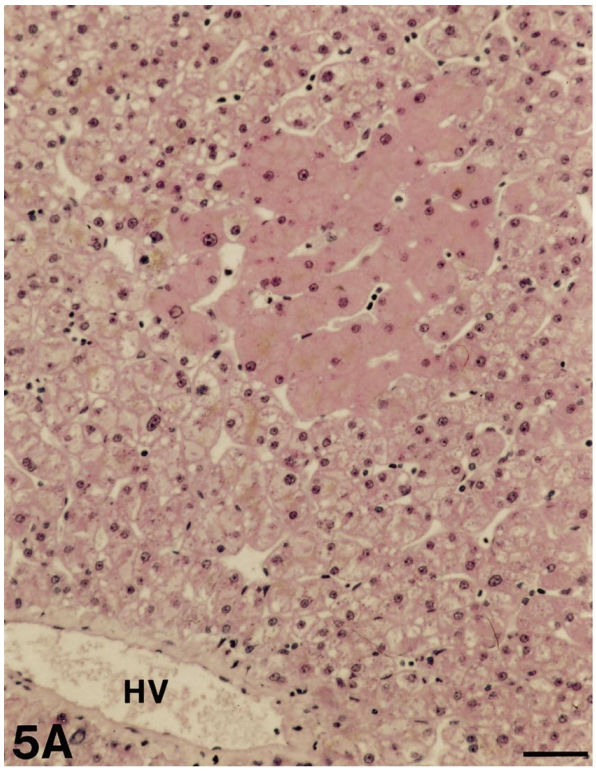
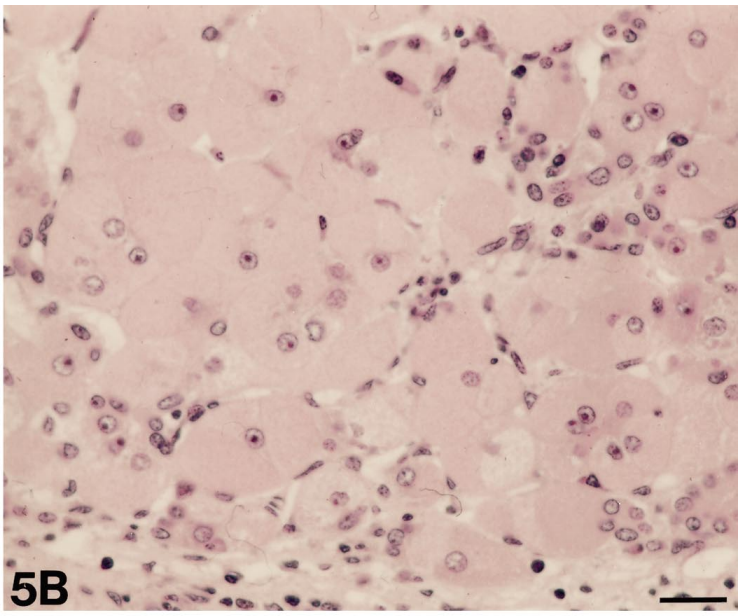


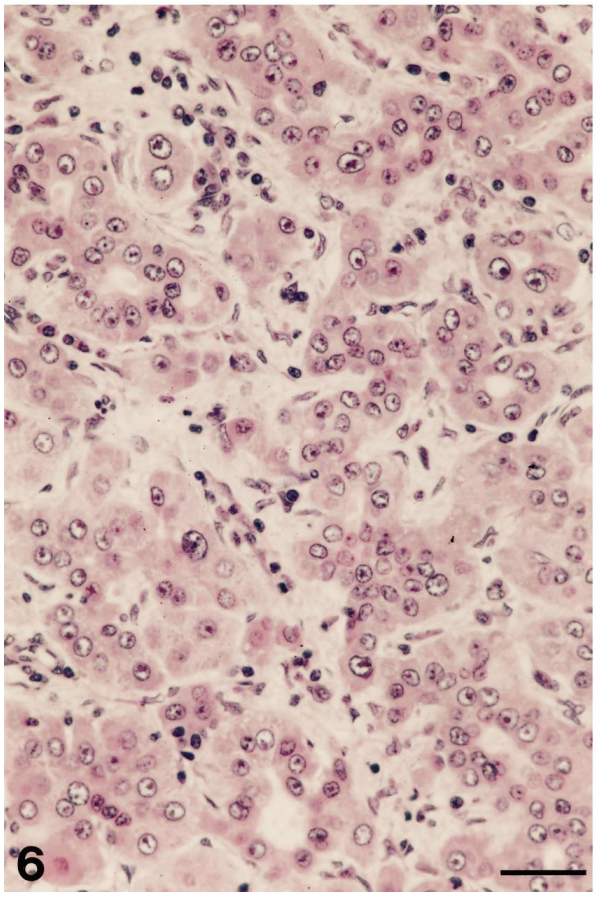
Fig. 4 Portions of cirrhotic livers **A–C** with coinfection of HBV and HCV, and **D** with HBV infection. **A** Small clear/basophilic mixed cell focus (*longer arrow*) with intrafocal small-cell change (FSCC), surrounded by several extrafocal enlarged hepatocytes (LCC, *shorter arrows*), H&E, ×160, *bar* 50 μm. **B** PCNA immunostaining in a section adjacent to **A**, demonstrating nuclear staining in the focus (*longer arrow*) and only cytoplasmic reactivity (*arrowhead*) in some large hepatocytes (*shorter arrows*) outside the focus. ×160, *bar* 50 μm. **C** Portion of clear/basophilic mixed cell focus with high-grade FSCC. H&E, ×160, *bar* 50 μm. **D** Portion of clear/basophilic mixed cell nodule with high-grade FSCC adjacent to large amphophilic hepatocytes (containing vacuolated nuclei, *arrow*) from a larger clear/amphophilic mixed cell nodule. H&E, ×220, *bar* 50 μm

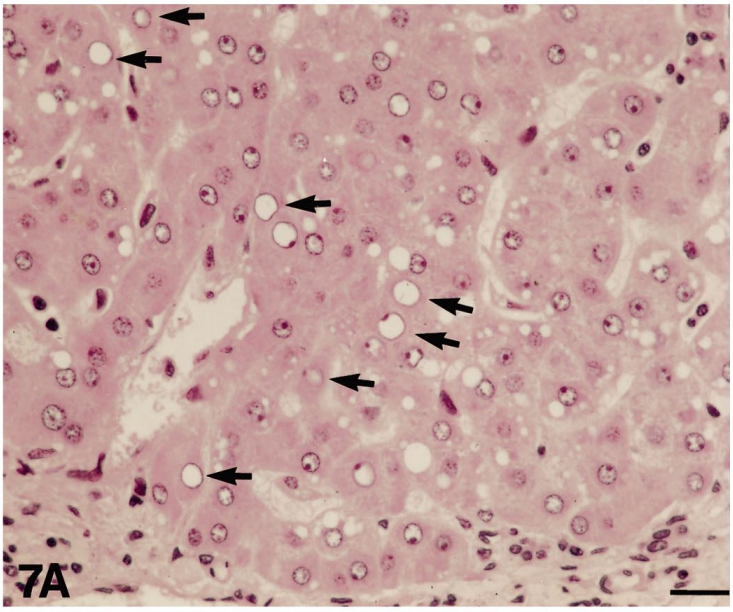
tical analysis. As shown in Fig. 1, the grade of MCF in cirrhotic livers in the high-risk group, particularly those bearing HCC, was significantly higher than in cirrhosis in the low-risk group. The grade of GSF in the HCC-bearing cirrhotic livers in the high-risk group was higher than in the livers in the low-risk group ($P < 0.05$). No significant difference was found between the high- and low-risk groups in the incidence and grade of oncocytic foci.

Most GSF are smaller than a hepatic lobule, located frequently in the periportal regions, but occasionally in other areas (Fig. 2A,B). They were composed of clear hepatocytes with relatively small nuclei and excessive storage of glycogen and sometimes also fat within the cytoplasm. In most GSF, the hepatocytes were larger than normal hepatocytes. The hepatic plates were one or two cells thick. A small number of large GSF showed pronounced expansive growth and formed clear cell nodules (Fig. 2C,D). The majority of MCF were composed mainly of glycogen-storing clear cells and glycogen-depleted amphophilic cells (MCFA; Fig. 3). There was a remarkable increase in both eosinophilia and basophilia in the cytoplasm of amphophilic hepatocytes. In addition, vacu-









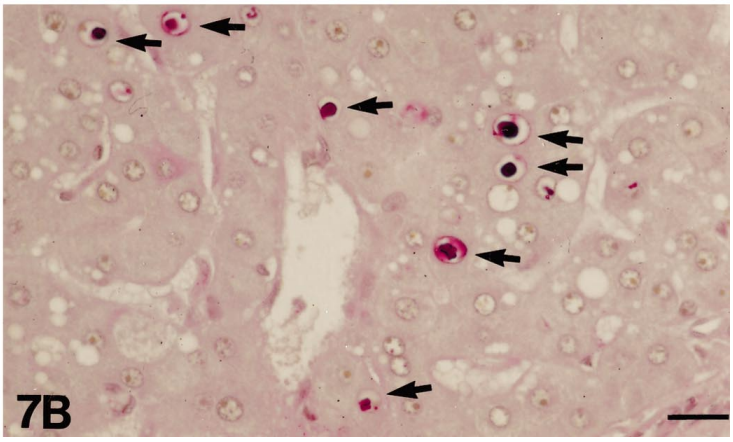


Fig. 5 **A** Oncocytic focus in a donor liver (*HV* hepatic venule) H&E, ×80, *bar* 100 µm. **B** Portion of oncocytic focus in a liver with HCC and HBV-associated cirrhosis. H&E, ×160, *bar* 50 µm

Fig. 6 Portion of basophilic cell focus with very pronounced intrafocal small-cell change and formation of glandular structures in a liver with HCC and HBV-associated cirrhosis. H&E, ×220, *bar* 50 µm

Fig. 7 **A, B** Portion of amphophilic cell focus with many glycogen-storing vacuolated nuclei (*arrows*) in a liver with HCV-associated cirrhosis, serial sections. **A** H&E, **B** Tri-PAS, ×160, *bar* 50 µm

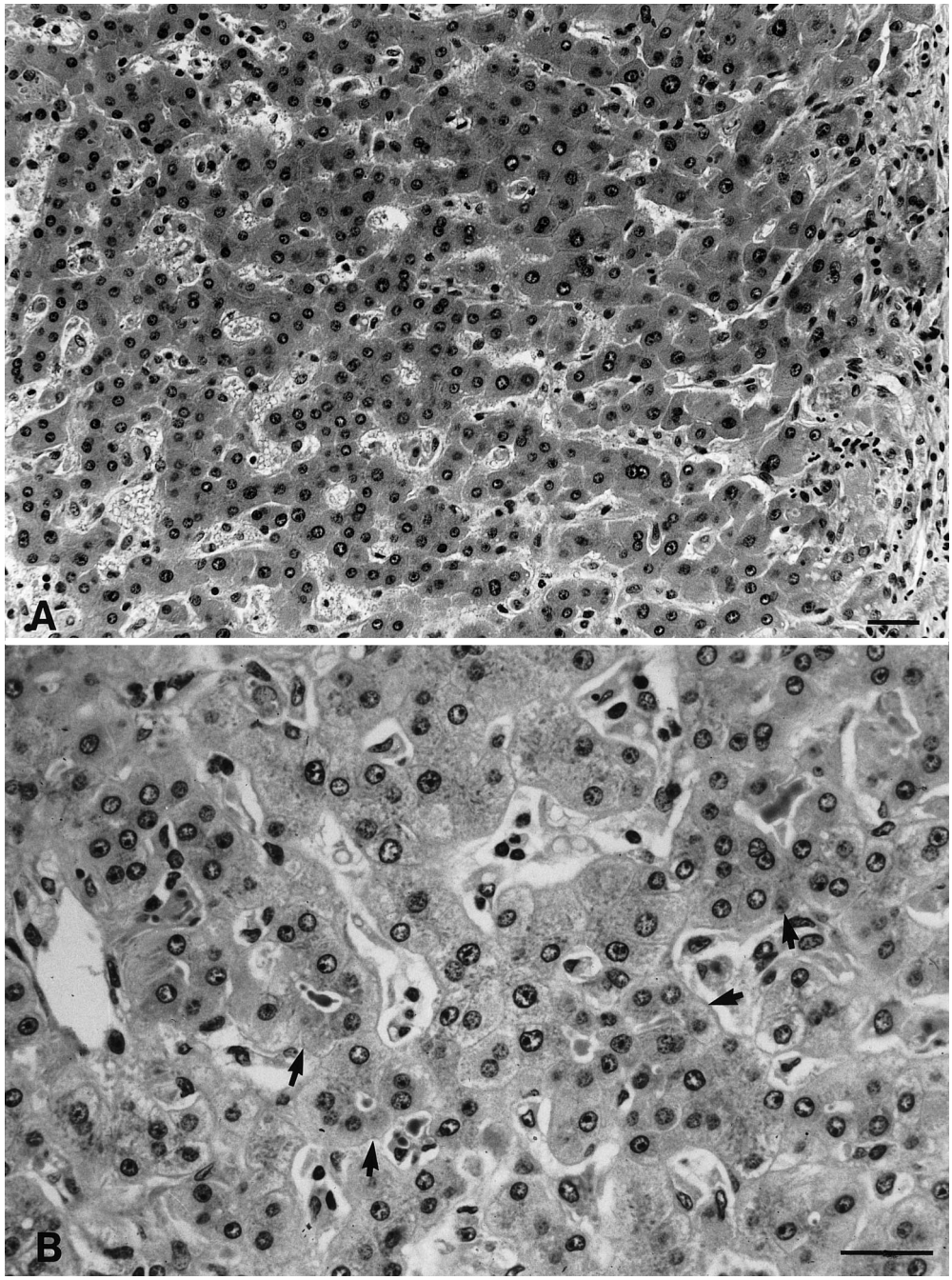


Fig. 8 **A, B** Small-cell change (SCC) in hepatocytes of diffuse type. H&E. **A** Diffuse SCC (*left*) in a specimen of primary sclerosing cholangitis, with transition to the parenchyma of normal appearance (*right*). ×150, *bar* 50 μm. **B** Diffuse SCC in a specimen of primary biliary cirrhosis, with thickened hepatic plates and formation of glandular structures (*arrows*) with bile plugs. ×290, *bar* 50 μm

olated nuclei storing glycogen (glycogenated nuclei) were often encountered in this type of hepatocyte. MCFB consisting of clear and basophilic hepatocytes (with or without amphophilic cells) were only observed in 11 of the 90 specimens from individuals with the high-risk or cryptogenic cirrhosis (Fig. 4A, C, D), sometimes demonstrating a nodule-in-nodule pattern within clear/amphophilic mixed cell nodules (Fig. 4D). Hepatocytes in MCF were more variable in size and shape than those in GSF. Single small hepatocytes with one or two small nuclei and two to five short cytoplasmic processes, described as small polygonal liver cells by Su et al. [62], were observed frequently in MCF. Most hepatic plates were two cells thick and connected imperceptibly with those of the surrounding parenchyma in small MCF. Larger MCF often showed pronounced expansive growth and formed nodular lesions (Fig. 3A, B, 4D).

Scattered hepatic oncocytes were encountered in 27% (30/111) of the cirrhotic specimens, being especially prevalent in those with HBsAg expression. The oncocytes were enlarged and packed with eosinophilic granules within glycogen-depleted cytoplasm (Fig. 5). Focal lesions composed predominantly or exclusively of oncocytes were rare and small, and often located in paraseptal regions (Fig. 5B). Oncocytic foci were observed in 16% (18/111) of the cirrhotic livers (Fig. 1). The incidence of oncocytic foci in HBV-associated cirrhosis (9/28) was higher than in cirrhosis from other causes (9/83)

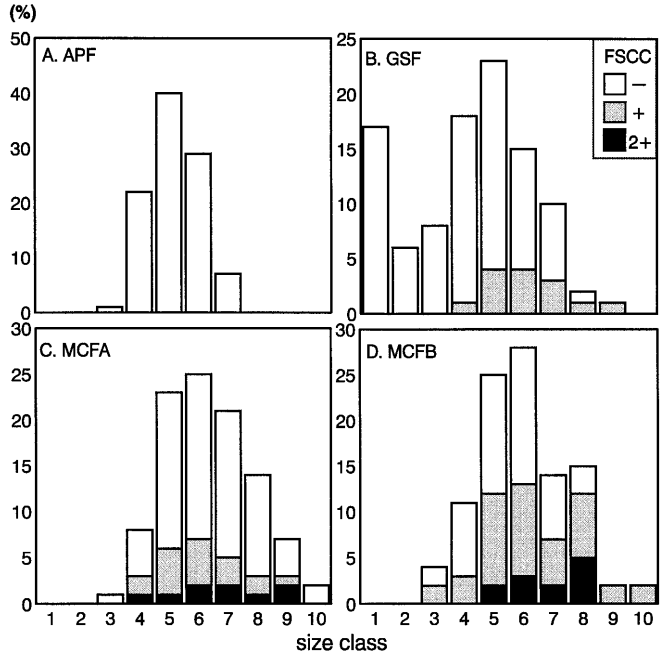


Fig. 9 Size class distribution of **A** amphophilic cell foci (*APF*), **B**, glycogen-storing foci (*GSF*), **C** clear/amphophilic (*MCFA*), and **D** clear/basophilic mixed cell foci (MCFB), as assessed by measuring the cross-sectional areas in square millimetres (size classes: *1* < 0.02; *2* 0.02–0.039; *3* 0.04–0.079; *4*, 0.08–0.159; *5* 0.16–0.319; *6* 0.32–0.639; *7* 0.64–1.279; *8* 1.28–2.559; *9* 2.56–5.119; *10* > 5.12) and expressed as percentages, associated with low-grade (+), high-grade (2+) intrafocal small-cell change (FSCC) or no FSCC (–)

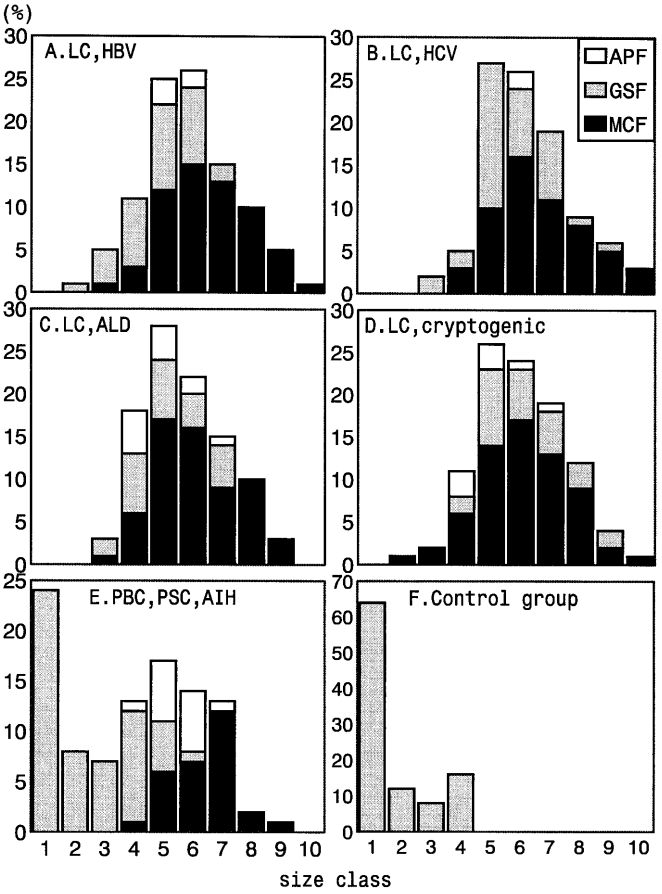


Fig. 10 A–F Size class distribution of amphophilic cell foci (*APF*), glycogen-storing foci (*GSF*) and mixed cell foci (*MCF*) in cirrhoses (*LC*) associated with **A** HBV infection, **B** HCV infection, and **C** alcoholic liver disease (*ALD*), **D** cryptogenic cirrhosis (**E**) primary biliary cirrhosis (*PBC*), **E** primary sclerosing cholangitis (PSC), cirrhosis due to autoimmune hepatitis (*AIH*), and **F** in the slightly disordered control livers, as assessed by measuring the cross-sectional areas in square millmetres (for definitions of size classes, see legend to Fig. 9) and expressed as percentages

($P < 0.01$). Single oncocytic foci were also found in a specimen of primary sclerosing cholangitis and a donor liver with slight perivenular fibrosis and fat storage (Fig. 5A). No mitotic figures were observed in these lesions. Although none of the oncocytic foci showed compression of the surrounding parenchyma, many oncocyte-like cancer cells were observed in two HCCs of the fibrolamellar type.

Basophilic cell foci were extremely rare, being found only in 2 cases of HBV-associated cirrhosis, 1 with and 1 without HCC. Three basophilic cell foci were observed in the former case (Fig. 6), and one in the latter.

In addition, areas composed exclusively of amphophilic hepatocytes (APF) were observed in 45% of cirrhotic livers (50/111), and in primary sclerosing cholangitis (5/9) and noncirrhotic livers bearing hepatocellular malignancies (3/5). APF were among the main types of FAH observed in primary sclerosing cholangitits, primary biliary cirrhosis, cirrhosis due to autoimmune hepatitis and alcoholic liver cirrhosis. There was no significant difference in grade of APF between the high- and low-risk cirrhotic liver groups (Fig. 1). The hepatic plates were one or two cells thick. Hepatocytes with glycogenated nuclei, distributed singly or in patches, were frequently observed in APF (Fig. 7). All APF were smaller than a hepatic lobule or a pseudolobule, and they were often located in periportal areas. No nodules composed exclusively of amphophilic hepatocytes were observed in this series. As we do not know their exact relation to the APF found in rodents, data for them are listed in Table 1 and Fig. 1 separately from other FAH.

Two types of SCC were identified. That observed only within FAH, designated as intrafocal SCC (FSCC), was found mainly in association with high-risk cirrhosis (Fig. 1). Within this group, the incidence and grade were significantly higher in HCC-bearing than in the HCC-free livers. FSCC was also observed in livers with cryptogenic cirrhosis (3/12) and in 2 noncirrhotic livers bearing HCC (Table 1). In most cases, FSCC appeared as small areas within FAH (Fig. 2E, 3, 4A), but occasionally it was found in a nodule-in-nodule pattern (Fig. 4D). Only low-grade FSCC were observed in GSF in 3 HCC-bearing and 4 HCC-free cirrhotic livers. In 6 of the 7 specimens, only 1 or a few lesions of this type were identified, but 33 GSF with FSCC were observed in 1 liver from a 42-year-old female patient with cryptogenic cirrhosis, and 14 of these 33 lesions met the criteria for nodules of altered hepatocytes. The hepatocytes in GSF with FSCC had many fine baso-

philic or acidophilic granules within their cytoplasm. The PAS reaction demonstrated a reduction of their glycogen content compared with GSF without FSCC. Hepatic plates in these GSF with FSCC were two to three cells thick (Fig. 2E). FSCC was often found in MCF (Fig. 3A, C, 4A, C, D), along with more pronounced nuclear atypia and structural disturbance of hepatic plates. Both mitotic figures and apoptotic bodies were observable in these foci, particularly in MCFB with FSCC. FSCC was identified in all four basophilic cell foci encountered (Fig. 6), but not in the APF or oncocytic foci.

Poorly-demarcated areas of SCC, all low grade, were also found in 9 cirrhotic or fibrotic livers with primary biliary cirrhosis, primary sclerosing cholangitis or cirrhosis due to autoimmune hepatitis (Table 1, Fig. 1). They were mainly located within large areas of extrafocal parenchyma, and were therefore designated diffuse SCC. These areas were irregular in shape and gradual transitions were often observed with parenchyma of normal appearance (Fig. 8A). The hepatic plates in areas with diffuse SCC were two cells thick, frequently with remarkably dilated canaliculi and glandular structure formation (Fig. 8B). MCFA, GSF or APF were included within these areas in 4 of the specimens.

LCC in hepatocytes was identified in 49.5% (55/111) of the specimens with cirrhosis (Table 1), its incidence being higher in cirrhosis due to HBV (75%, 21/28) than in cirrhosis caused by HCV (34.8%, 8/23; $P < 0.005$) or other causative factors (43.3%, 26/60; $P < 0.01$). Within the high-risk group, LCC was more pronounced in the livers bearing HCC than in those without HCC. However, no significant difference in the incidence and grade of LCC was observed between HCC-free cirrhotic livers of the high- and the low-risk groups (Fig. 1). LCC was found mainly in extrafocal parenchyma (Fig. 4A), especially in HBsAg-positive areas. It was also seen in some FAH, in particular those containing amphophilic hepatocytes (Fig. 4D). Here it was frequently in a scattered pattern, but it was occasionally seen in clusters. However, none of these lesions were well-demarcated or gave rise to any distinct compression of the surrounding tissue.

Altogether, 831 focal lesions were identified for estimation of size and cell density, from 78 specimens containing FAH. They included 306 GSF, 72 APF, 393 MCFA and 60 MCFB. FSCC was identified in 44 (14.4%) GSF, 99 (25.2%) MCFA and 31 (51.7%) MCFB (Fig. 9). The grade of FSCC was higher in MCFB than in MCFA ($P < 0.0005$) or in GSF ($P < 0.0001$). In total, 204 of the 831 FAH met the criteria for nodules of altered hepatocytes. Among these nodules, 178 were of mixed cell type, 17 of clear cell type with FSCC, and 9 of clear cell type without FSCC. The frequency of nodule formation by MCFA (160/393, 40.7%) and MCFB (18/60, 30.0%) was higher than for GSF (26/306, 8.5%; $P < 0.001$). GSF with FSCC (17/44, 38.6%) were more frequently dignosed as nodules than those without FSCC (9/262, 3.4%; $P < 0.0001$).

As shown in Fig. 9, the average size (cross-sectional area) was greater for MCFA and MCFB than for GSF without FSCC ($P < 0.0001$). GSF with FSCC were larger than those without FSCC ($P < 0.0001$) and comparable to MCF. However, no significant difference was found between the MCFA with FSCC and without FSCC. MCFB with FSCC tended to be larger than those without FSCC, but the difference did not attain statistical significance. APF were larger than GSF without FSCC ($P < 0.001$), but smaller than MCFA ($P < 0.0001$) and MCFB ($P < 0.05$). As shown in Fig. 10, GSF in the control livers and in primary biliary cirrhosis, primary sclerosing cholangitis and cirrhosis due to autoimmune hepatitis were significantly smaller than in cirrhosis due to other causes ($P < 0.0001$). GSF in HCV-associated cirrhosis were significantly larger than those in HBV-associated cirrhosis ($P < 0.05$). GSF in cryptogenic cirrhosis were also larger than in HBV-associated cirrhosis ($P < 0.0001$), in line with the high proportion with FSCC (33/44). No significant difference was found in the average size of MCF in different liver diseases, but MCF in the high-risk group and cryptogenic cirrhoses were more variable in size.

Data for proliferative activity, of different cell populations as assessed by PCNA-LI, are shown in Fig. 11. The PCNA-LI of hepatocytes in extrafocal parenchyma of all explanted and resected livers from patients with severe chronic liver disease was higher than the donor liver value ($P < 0.0001$). The extrafocal hepatocytes in primary biliary cirrhosis, primary sclerosing cholangitis and cirrhosis due to autoimmune hepatitis ($P < 0.001$), cryptogenic cirrhosis ($P < 0.05$) and alcoholic cirrhosis ($P < 0.05$) demonstrated more proliferation than those in HBV-associated cirrhosis. This might be attributed to the cytoplasmic storage of HBsAg and preferential emergence of LCC in the extrafocal parenchyma in the latter case. The labelling index of the extrafocal parenchyma in HCV-associated cirrhosis tended to be higher than with HBV-associated cirrhosis, but the difference did not attain statistical significance. PCNA-LI in both GSF and APF were as low as in donor livers, and significantly lower than in the extrafocal parenchyma ($P < 0.0005$). Hepatocytes in MCFA and MCFB were more proliferative than those in the extrafocal parenchyma ($P < 0.0001$). The labelling index for MCFB was higher than that for MCFA ($P < 0.01$). Very few nuclei of the hepatocytes in LCC were labelled by PC10, but cytoplasmic immunoreactivity, localized in certain areas, was encountered in these cells in some cirrhotic livers (Fig. 4B)

PCNA-LIs for GSF, MCFA and MCFB with FSCC were found to be greatly increased compared with their counterparts without FSCC ($P < 0.0001$; Fig. 2F, 4B). Hepatocytes in MCFA and MCFB with FSCC were more proliferative than those in GSF with FSCC ($P = 0.0001$). PCNA-LIs for GSF and MCFA included in diffuse SCC were lower than those with FSCC ($P = 0.002$), and higher than those without SCC ($P < 0.005$). However, no significant difference in PCNA-LI was observed between the extrafocal parenchyma with and without diffuse SCC.

The cell density in donor livers ranged from 1181 to 1890 nucleated hepatocytes/mm^2, and was more variable

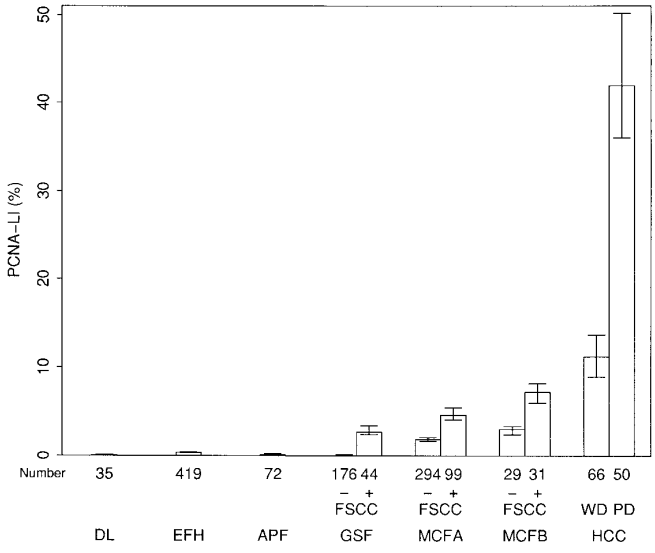


Fig. 11 PCNA labelling indices (*PCNA-LI*) in donor livers (*DL*), extrafocal hepatocytes (*EFH*) of livers with advanced chronic diseases, hepatic focal lesions including amphophilic cell foci (*APF*), glycogen-storing foci (*GSF*), clear/amphophilic (*MCFA*) and clear/basophilic mixed cell foci (*MCFB*) with (+) or without (–) intrafocal small-cell change (*FSCC*), and in well- (*WD*) and poorly differentiated (*PD*) HCC, with values given as median ± 95% confidence limit

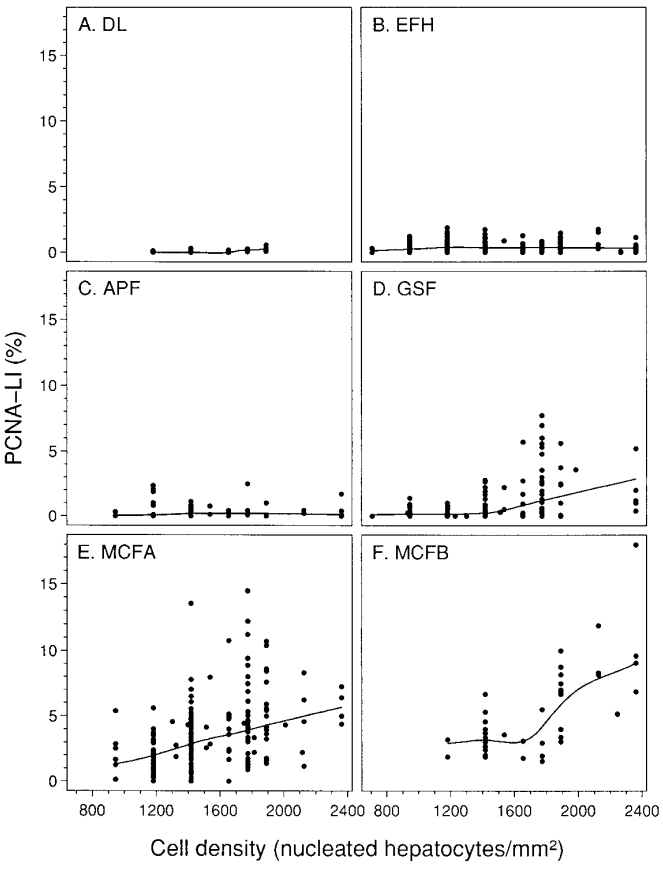


Fig. 12 **A–F** Regression analysis between PCNA labelling indices (*PCNA-LI*) and cell densities in **A** donor livers (*DL*), **B** extrafocal hepatocytes (*EFH*), **C** amphophilic cell foci (*APF*), **D** glycogen-storing foci (*GSF*), **E** clear/amphophilic (*MCFA*) and **F** clear/basophilic mixed cell foci (*MCFB*), with positive correlations observed in **D**, **E** and **F**, but not in **A**, **B** and **C**

in FAH and the extrafocal parenchyma of livers with chronic diseases. Regression analysis was made between PCNA-LI and the cell density, ranging from 500 to 2400 nucleated hepatocytes/mm^2, where the data allowed. Positive correlations were found in MCFB, MCFA and GSF, but not in APF, extrafocal parenchyma and donor livers (Fig. 12).

Discussion

Although substantial progress has been made in our understanding of the pathogenesis of HCC, many aspects are still obscure. Cirrhosis itself is believed to be a precancerous condition. However, considerable variation in susceptibility exists among cirrhoses due to different causative factors. Cirrhosis caused by chronic infection with HBV or HCV or by chronic alcohol abuse is associated with a high risk of developing HCC [7, 15, 18]. In contrast, primary biliary cirrhosis, primary sclerosing cholangitis, Budd–Chiari syndrome, Byler's disease, Wilson's disease and genetic haemochromatosis have been reported to be lower risk conditions [25, 28, 41, 50, 52, 53, 58, 67]. The occurrence of about 20% of HCCs in noncirrhotic livers, as demonstrated in this and a number of previous studies [12, 24, 55], indicates that cirrhosis is not an obligatory prerequisite for HCC development.

Large-cell change of hepatocytes was thought to be a precancerous change by Anthony et al. [3] and has attracted a great deal of attention for more than 2 decades [14, 17], but accumulated data from many authors do not support this hypothesis [22, 37, 42, 51, 63, 68]. In the present study, LCC was observed in 49.5% (55/111) of the cirrhotic livers, its incidence and grade being higher in those bearing HCC. However, its frequent occurrence in primary sclerosing cholangitis (5/9), and even in 3 livers with subacute fulminant hepatitis, its scattered distribution, and its low cell proliferative activity argue against a precancerous nature. LCC may in fact result from a disturbance in hepatocellular replication, as previously suggested by Altmann [2] and Su et al. [63]. Aberrant cytoplasmic PCNA-immunoreactivity was observed in hepatocytes with LCC in some cirrhotic livers, as reported earlier for some Reed-Sternberg cells in Hodgkin lymphoma [13], suggesting an abnormality in transport of PCNA between the cytoplasm and nucleus.

Based mainly on its phenotypic similarities to well-differentiated HCC, SCC has been considered to be a precancerous change [29, 44, 63, 68, 74]. The incidences of SCC in cirrhotic livers have been reported to be 18.5% in HCC-bearing and 2.4% in HCC-free biopsies and necropsies by Watanabe et al. [68], and 86.11% in HCC-bearing and 54% in HCC-free biopsy specimens by Zhao et al. [74]. It is not clear whether the difference between these two reports is due to differences in the morphological criteria applied or in the cases studied. Zhao et al. [74] divided SCC into "small regenerating liver cells" and "small liver cell dysplasia", and regarded only the latter as precancerous. Nakanuma and Hirata [49] de-

scribed a high percentage of SCC in primary biliary cirrhosis in the fibrotic stage and regarded it as a reactive change unrelated to HCC. In this study, we examined 163 cases of explanted and resected liver specimens, and two types of SCC, namely FSCC and diffuse SCC, were identified. Diffuse SCC was observed only in primary biliary cirrhosis, primary sclerosing cholangitis and cirrhosis due to autoimmune hepatitis, and may be identical to the lesion described by Nakanuma and Hirata in primary biliary cirrhosis [49]. The proliferative activity of extrafocal hepatocytes with and without diffuse SCC was not significantly different. Hepatocytes in the FAH included in diffuse SCC were found not to proliferate as readily as those in FAH with FSCC. It is, thus, unlikely that diffuse SCC is a preneoplastic lesion. It may be an adaptive change associated with cholestasis and periportal hepatocellular injuries. However, FSCC was found in 25.2% (28/111) of the cirrhotic livers, mainly in those associated with HBV or HCV infection or alcoholic liver disease. Their highly preferential occurrence in HCC-bearing livers (52.8%, 19/36) and their positive correlation with an elevated proliferation and nodular transformation of FAH strongly indicate that FSCC represent a late stage of preneoplasia and may be regarded as a precancerous lesion.

FAH, mainly clear cell foci, have been observed in human livers as incidental findings by several authors [2, 5, 16, 30, 35, 42]. Recently, Bannasch et al. [11] described FAH storing glycogen excessively in all HCC-bearing livers examined, and in 50% of cirrhotic livers without HCC in a series of 69 explanted and resected human livers. In this study, we extend these observations and provide, for the first time, evidence for a high incidence of different types of FAH in various kinds of liver diseases. FAH of clear and mixed cell type were observed in almost all livers bearing HCC, and in chronic liver diseases without HCC but at a lower frequency. With HCC-free cirrhosis, the incidence of FAH was found to be significantly higher in the high-risk than in the low-risk group. These results strongly support the view that FAH, particularly GSF and all types of MCF, and the resulting hyperproliferative nodular lesions, are associated with HCC development in humans [2, 11].

It has been pointed out that FAH in rodent liver have different phenotypes and that these phenotypes tend to change sequentially during hepatocarcinogenesis [7]. GSF represent early lesions, with the potential to progress to more advanced glycogen-poor basophilic cell lesions through MCF or some other intermediates [10]. An inverse correlation between glycogen accumulation and cell proliferation has been observed for this process [73]. In our human specimens, GSF were observed in a broad spectrum of liver diseases, even in some donor livers with perivenular fibrosis indicative of chronic damage, perhaps from alcohol abuse [60], and these GSF without FSCC had a lower proliferative activity than extrafocal parenchyma. However, the GSF observed in cirrhotic livers were significantly larger and more variable in size than those in the slightly disordered control livers (see Fig. 10). Furthermore, those GSF with FSCC were phenotypically similar to the intermediate cell foci observed in rodents [10] and demonstrated a higher PCNA labelling index and more frequent nodular transformation, meaning that these lesions are a more advanced stage of GSF. The proliferation of hepatocytes in GSF and in extrafocal parenchyma of human livers was found to be different, namely lower and higher, respectively, than that reported for the corresponding tissue compartments of rodents treated with *N*-nitrosomorpholine [73] or the peroxisome proliferator Wy-14, 643 [47]. This discrepancy reflects the relatively stable conditions of animal livers, studied several weeks or months after withdrawal of the carcinogen, as opposed to human livers affected by advanced chronic disease. Hepatocytes in the human liver can proliferate in response to frequent regenerative local stimuli, such as spot or piecemeal necrosis in chronic viral hepatitis [63]. However, the response to such local stimuli seems to be muted in most GSF discovered in our material. It is evident, as observed in several animal models [23, 61, 73], that growth control of hepatocytes is altered in FAH. We demonstrated a positive correlation between the hyperproliferation of hepatocytes and a reduction in cell size and the resulting increase in cell density in GSF, MCFA and MCFB, but not in extrafocal parenchyma (see Fig. 12).

The finding of MCF mainly in the livers with high-risk or cryptogenic cirrhosis indicates that these are more advanced precursor lesions in man, in line with earlier observations for experimental animals [10]. Considering the preferential emergence in cirrhotic livers of the high-risk group, their unequivocally elevated proliferative activity and the resulting large size with frequent nodular transformation, we suggest that MCF are endowed with the potential to progress to HCC in humans, as previously shown in rats [27, 69]. The fact that FSCC was observed in 25% of MCFA and 52% of MCFB, but only in 14% of GSF also suggests that MCF are more unstable. Close association between FSCC and more advanced FAH indicates that some FAH may progress to HCC through FSCC. The presence of FSCC in all basophilic cell foci observed implies that this type of FAH is a late preneoplastic lesion, in spite of its rarity. We think that uneven growth of liver parenchyma, caused by the emergence of different types of FAH with an altered growth control, is a crucial step in HCC development.

Small areas or clusters of hepatic oncocytes (oncocytic foci) were also observed, mainly in cirrhotic livers (18/111), with a preferential occurrence in HBV-associated cirrhosis (9/28). Similar lesions have earlier been described by other authors in HBsAg-positive liver tissues and in association with other liver diseases [32, 45]. Scattered oncocytes have been observed in the livers of woodchucks infected with woodchuck hepatitis virus with and without administration of aflatoxin B_1 and thus appear to be involved in the process of hepatocarcinogenesis in this animal model [9]. Benign and malignant oncocytic neoplasms have been found in many organs [19], including human liver [2, 19, 56, 59]. A large quantity of oncocyte-like cancer cells was also observed in the fibrolamellar carcinoma in this study. It therefore

seems reasonable to consider oncocytic foci a preneoplastic lesion.

APF have been demonstrated to be preneoplastic lesions in the livers of rodents exposed to different hepatocarcinogenic chemicals [7, 48] or to hepadnaviruses [9]. In this study, we observed APF in 45% of the cirrhotic livers. Although both the GSF without FSCC and APF were less proliferative than the surrounding liver parenchyma, APF were on average the larger of the two. However, they were smaller than MCF including MCFA, and did not show nodular transformation or FSCC. In spite of the striking similarity in morphological phenotype between APF in the human liver and the corresponding lesions in rodent liver, it is difficult to assess their possible preneoplastic nature. APF might indeed be a precursor of MCFA. However, we cannot at present exclude the possibility that they might at least in part develop through phenotypic reversion of more advanced lesions. Clearly, more work has to be done to clarify the biological behaviour of APF in the human liver.

It has been shown by several authors using different experimental approaches that most of the FAH and hepatocellular neoplasms examined in rodents are clonal in origin [66, 70]. It has been accepted in rodents that the advanced focal lesions showing definite compression of the surrounding parenchyma are neoplastic lesions (hepatocellular adenomas) [33]. Criteria for the identification of these neoplastic lesions in human cirrhotic livers, however, remain to be established. Our present results showed that most nodular lesions developed from MCF or GSF with FSCC, and that hepatocytes within these nodules were more proliferative than those in the extrafocal parenchyma and hence represented hyperproliferative populations. At least some of these nodules may already be neoplastic. Molecular analyses of HBV DNA integration patterns [65, 72] and methylations of the polymorphic X-chromosome-linked phosphoglycerate kinase [1, 43] and androgen receptor [31] gene in female patients has demonstrated a clonal origin for most hepatocellular neoplasms and some cirrhotic nodules. Further work is needed to clarify whether these nodules correspond to nodules of altered hepatocytes.

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References

1. Aihara T, Noguchi S, Sasaki Y, Nakano H, Imaoka S (1994) Clonal analysis of regenerative nodules in hepatitis C virus-induced liver cirrhosis. Gastroenterol 107:1805–1811
2. Altmann HW (1994) Hepatic neoformations. Pathol Res Pract 190:513–577
3. Anthony PP, Vogel CL, Barker LF (1973) Liver cell dysplasia: a premalignant condition. J Clin Pathol 26:217–223
4. Armitage P (1955) Test for linear trend in proportions and frequencies. Biometrics 11:375–386
5. Balázs M (1976) Lichtmikroskopische Untersuchungen in einem Fall von primärem Leberkarzinom im Säuglingsalter. Zentralbl allgem Pathol 120:3–13
6. Bannasch P (1986) Preneoplastic lesions as end points in carcinogenicity testing. I. Hepatic preneoplasia. Carcinogenesis 7:849–852
7. Bannasch P (1996) Pathogenesis of hepatocellular cacinoma: sequential cellular, molecular, and metabolic changes. In: Boyer JL, Ockner RK (eds) Progress in liver diseases, vol 14. Saunders, Philadelphia, pp 161–197
8. Bannasch P, Mayer D, Hacker HJ (1980) Hepatocellular glycogenosis and hepatocarcinogenesis. Biochim Biophys Acta 605:217–245
9. Bannasch P, Imani Khoshkhou N, Hacker HJ, Radaeva S, Mrozek M, Zillmann U, Kopp-Schneider A, Haberkorn U, Elgas M, Tolle T, Roggendorf M, Toshkov I (1995) Synergistic hepatocarcinogenic effect of hepadnaviral infection and dietary aflatoxin B_1 in woodchucks. Cancer Res 55:3318–3330
10. Bannasch P, Zerban H, Hacker HJ (1997) Foci of altered hepatocytes, rat. In: Jones TC, Popp J, Mohr U (eds) Monographs on pathology of laboratory animals. Digestive system. Springer, Berlin Heidelberg New York Tokyo, pp 3–37
11. Bannasch P, Jahn U-R, Hacker HJ, Su Q, Hofmann W, Pichlmayr R, Otto G (1997) Focal hepatic glycogenosis: a putative preneoplastic lesion associated with neoplasia and cirrhosis in explanted human livers. Int J Oncol 10:261–268
12. Bartoloni S, Omer F, Giannini A, Napoli P (1984) Hepatocellular carcinoma and cirrhosis: a review of their relative incidence in a 25-year period in the Florence area. Hepatogastroenterology 31:215–217
13. Benjamin DR, Gown AM (1991) Aberrant cytoplasmic expression of proliferating cell nuclear antigen in Hodgkin's disease. Am J Clin Pathol 15:764–768
14. Borzio M, Bruno S, Mels GC, Ramella G, Borzio F, Leandro G, Servida E, Podda M (1995) Liver cell dysplasia is a major risk factor for hepatocellular carcinoma in cirrhosis: a prospective study. Gastroenterology 108:812–817
15. Bosch FX, Muñoz N (1989) Epidemiology of hepatocellular carcinoma. In: Bannasch P, Keppler D, Weber G (eds) Liver cell carcinoma. Kluwer Academic, Dordrecht Boston London, pp 3–14
16. Cain H, Kraus B (1977) Entwicklungsstörungen der Leber und Leberkarzinom im Säuglings- und Kindesalter. Dtsch Med Wochenschr 102:505–509
17. Canne-Carrié N, Chastang C, Munz C, Pateron D, Sibony M, Déng P, Trinchet J-C, Callard P, Guettier C, Beaugrand M (1996) Predictive score for the development of hepatocellular carcinoma and additional value of liver cell dysplasia in western patients with cirrhosis. Hepatology 23:1112–1118
18. Caselmann WH, Alt M (1996) Hepatitis C virus infection as a major risk factor for hepatocellular carcinoma. J Hepatol 24 [Suppl 2]:61–66
19. Chang A, Harawi SJ (1992) Oncocytes, oncocytosis, and oncocytic tumors. Pathol Annu 27:263–304
20. Cleveland WS, Grosse E (1991) Computational methods for local regression. Statistics and Computing 1:47–62
21. Cochran WG (1954) Some methods for strengthening the common χ^2 test. Biometrics 10:417–454
22. Cohen C, Berson SD (1986) Liver cell dysplasia in normal, cirrhotic and hepatocellular carcinoma patients. Cancer 57:1535–1538
23. Columbano A, Ledda-Columbano GM, Rao PM, Rajalakshmi S, Sarma DSR (1984) Occurrence of cell death (apoptosis) in preneoplastic and neoplastic liver cells: a sequential study. Am J Pathol 116:441–446
24. Craig JR, Peters RL, Edmondson HA (1989) Tumors of the liver and intrahepatic bile ducts. Armed Forces Institute of Pathology, Washington DC, pp 6–7
25. Deugnier YD, Charalambous P, Le Quilleuc D, Turlin B, Searle J, Brissot P, Powell LW, Halliday JW (1993) Preneoplastic significance of hepatic iron-free foci in genetic haemochromatosis: a study of 185 patients. Hepatology 18:1363–1369

26. Edmondson HA, Steiner PE (1954) Primary carcinoma of the liver: a study of 100 cases among 48900 necropsies. Cancer 7:462–503
27. Enzmann H, Bannasch P (1987) Potential significance of phenotypic heterogeneity of focal lesions at different stages in hepatocarcinogenesis. Carcinogenesis 8:1607–1612
28. Eriksson S, Carlson J, Velez R (1986) Risk of cirrhosis and primary liver cancer in alpha-1-antitrypsin deficiency. N Engl J Med 314:736–739
29. Ferrel LD, Crawford JM, Dhillon AP, Scheuer PJ, 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
30. Fischer G, Hartmann H, Droese M, Schauer A, Bock KW (1986) Histochemical and immunohistochemical detection of putative preneoplastic liver foci in women after long-term use of oral contraceptives. Virchows Arch [B] 50:321–337
31. Gaffey MJ, Iezzoni JC, Weiss LM (1996) Clonal analysis of focal nodular hyperplasia of the liver. Am J Pathol 148:1089–1096
32. Gerber MA, Thung SN (1981) Hepatic oncocytes. Incidence, staining characteristics, and ultrastructural features. Am J Clin Pathol 75:498–503
33. Goodman DG, Maronpot RR, Newberne PM, Popp JA, Squire RA (1994) Proliferative and selected other lesions of the liver in rats. In: Guides for toxicologic pathology. STP/ARP/AFIP, Washington DC, pp 1–24
34. Harris CC (1995) 1995 Deichmann Lecture – p53 tumor suppressor gene: at the crossroads of molecular carcinogenesis, molecular epidemiology and cancer risk assessment. Toxicol Lett 82/83:1–7
35. Hirota N, Hamazaki M, Williams GM (1982) Resistance to iron accumulation and presence of hepatitis B surface antigen in preneoplastic and neoplastic lesions in human haemochromatotic livers. Hepatogastroenterol 29:49–51
36. Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Statist 6:65–67
37. Hytigoglou P, Theise ND, Schwartz M, Mor E, Miller C, Thung SN (1995) Macroregenerative nodules in a series of adult cirrhotic liver explants: issues of classification and nomenclature. Hepatology 21:703–708
38. Imaida K, Tatematsu M, Kato T, Tsuda H, Ito N (1989) Advantages and limitations of stereological estimation of placental glutathione *S* -transferase-positive rat liver foci by computerized three-dimensional reconstruction. Jpn J Cancer Res 80:326–330
39. International Working Party (1995) Terminology of nodular hepatocellular lesions. Hepatology 22:983–993
40. Ishak KG, Anthony PP, Sobin LH (1994) Histological typing of tumours of the liver. In: World Health Organization, International histological classification of tumours, 2nd edn. Springer, Berlin Heidelberg New York, pp 9–22
41. Ismail T, Angrisani L, Hubscher S, McMaster P (1991) Hepatocellular carcinoma complicating primary sclerosing cholangitis. Br J Surg 78:360–361
42. Karhunen PJ, Penttilä A (1987) Preneoplastic lesions of human liver. Hepatogastroenterology 34:10–15
43. Kawai S, Imazeki F, Yokosuka O, Ohta M, Shiina S, Kato N, Omata M (1995) Clonality in hepatocellular carcinoma: analysis of methylation pattern of polymorphic X-chromosome-linked phosphoglycerate kinase gene in females. Hepatology 22:112–117
44. Le Bail B, Belleannée G, Bernard P-H, Saric J, Balabaud C, Bioulac-Sage P. (1995) Adenomatous hyperplasia in cirrhotic livers: histological evaluation, cellular density, and proliferative activity of 35 macronodular lesions in the cirrhotic explants of 10 adult French patients. Hum Pathol 26:897–906
45. Lefkowitch JH, Arborgh BÅ M, Scheuer PJ (1980) Oxyphilic granular hepatocytes: mitochondria-rich liver cells in hepatic disease. Am J Clin Pathol 74:432–441
46. Lehmann EL (1975) Nonparametrics: statistical methods based on ranks. Holden and Day, San Francisco
47. Marsman DS, Popp JA (1994) Biologic potential of basophilic hepatocellular foci and hepatic adenoma induced by the peroxisome proliferator, Wy-14, 643. Carcinogenesis 15:111–117
48. Metzger C, Mayer D, Hoffmann H, Bocker T, Hobe G, Benner A, Bannasch P (1995) Sequential appearance and ultrastructure of amphophilic cell foci, adenomas, and carcinomas in the liver of male and female rats treated with dehydroepiandrosterone. Toxicol Pathol 23:591–605
49. Nakanuma Y, Hirata K (1993) Unusual hepatocellular lesions in primary biliary cirrhosis resembling but unrelated to hepatocellular carcinoma. Virchows Arch [A] Path Anat 422:17–23
50. Nakanuma Y, Terada T, Doishita K, Miwa A (1990) Hepatocellular carcinoma in primary biliary cirrhosis: an autopsy study. Hepatology 11:1010–1016
51. Nakashima T, Okuda K, Kojiro M, Jimi A, Yamaguchi R, Sakamoto K, Ikari T (1983) Pathology of hepatocellular carcinoma in Japan, 232 consecutive cases autopsied in 10 years. Cancer 51:863–877
52. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G (1985) Survival and causes of death in cirrhotic and noncirrhotic patients with primary haemochromatosis. N Engl J Med 313:1256–1262
53. Okuda K (1982) Membranous obstruction of the inferior vena cava: etiology and relation to hepatocellular carcinoma. Gastroenterology 82:376–379
54. Okuda K (1992) Hepatocellular carcinoma: recent progress. Hepatol 15:948–963
55. Okuda K, Fujimoto I, Hanai A, Urano Y (1987) Changing incidence of hepatocellular carcinoma in Japan. Cancer Res 47:4967–4972
56. Payne CM, Nagle RB, Paplanus SH, Graham AR (1986) Fibrolamellar carcinoma of liver: a primary malignant oncocytic carcinoid? Ultrastruct Pathol 10:539–552
57. Pitot HC (1990) Altered hepatic foci: their role in murine hepatocarcinogenesis. Annu Rev Pharmacol Toxicol 30:465–500
58. Polio J, Enriquez RE, Chow A, Wood WM, Atterbury CE (1989) Hepatocellular carcinoma in Wilson's disease. J Clin Gstroenterol 11:220–224
59. Salisbury JR, Portmann BC (1987) Oncocytic liver cell adenoma. Histopathology 11:533–539
60. Savolainen V, Perola M, Lalu K, Pentttilä A, Virtanen I, Karhunen PJ (1995) Early perivenular fibrogenesis – precirrhotic lesions among moderate alcohol consumers and chronic alcoholics. J Hepatol 23:524–531
61. Schulte-Hermann R, Bursch W, Grasl-Kraupp B, Mülauer L, Ruttkay-Nedecky B (1995) Apoptosis and multistage carcinogenesis in rat liver. Mutat Res 333:81–87
62. Su Q, Liu YF, Zhang, JF, Zhang SX, Yang JJ (1994) Expression of insulin-like growth factor II in hepatitis, cirrhosis and hepatocellular carcinoma: its relationship with hepatitis B virus antigen expression. Hepatology 20:788–799
63. Su Q, Zhang SX, Yang SJ, Liu J, Liu YF (1995) Precancerous changes in hepatitis B and liver cirrhosis: a morphological and immunohistochemical study. J Fourth Milit Med Univ 16:85–88
64. Tabor E (1994) Tumor suppressor genes, growth factor genes, and oncogenes in hepatitis B virus-associated hepatocellular carcinoma. J Med Virol 42:357–365
65. Tsuda H, Hirohashi S, Shimosato Y, Terada M, Hasegawa H (1988) Clonal origin of atypical adenomatous hyperplasia of the liver and clonal identity with hepatocellular carcinoma. Gastroenterology 95:1664–1666
66. Tsunashima K, Endo Y, Asakura H, Kanda H, Nomura K, Kitagawa T, Kominami R (1996) A novel clonal assay for the mouse: application to hepatocellular carcinomas induced with diethylnitrosamine. Mol Carcinog 15:33–37
67. Ugarte N, Gonzalez-Crussi F (1981) Hepatoma in siblings with progressive familial cholestatic cirrhosis of childhood. Am J Clin Pathol 76:172–177
68. Watanabe S, Okita K, Harada T, Kodama T, Numa Y, Takemoto T, Takahashi T (1983) Morphologic studies of the liver cell dysplasia. Cancer 51:2197–2205

69. Weber E, Bannasch P (1994) Dose and time dependence of the cellular phenotype in rat hepatic preneoplasia and neoplasia induced by continuous oral exposure to *N* -nitrosomorpholine. Carcinogenesis 15:1235–1240
70. Weinberg WC, Ng YK, Iannaccone PM (1992) Clonal analysis of hepatic neoplasms by mosaic pattern. In: Sirica AE (ed) The role of cell types in hepatocarcinogenesis. CRC Press, Boca Raton Ann Arbor London, pp 29–53
71. Williams GM (1980) The pathogenesis of rat liver cancer caused by chemical carcinogens. Biochim Biophys Acta 605:167–189
72. Yasui H, Hino O, Ohtake K, Machinami R, Kitagawa T (1992) Clonal growth of hepatits B-integrated hepatocytes in cirrhotic liver nodules. Cancer Res 52:6810–6814
73. Zerban H, Radig S, Kopp-Schneider A, 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
74. Zhao M, Zhang NX, Du ZY, Laissue JA, Zimmermann A (1994) Three types of liver cell dysplasia (LCD) in small cirrhotic nodules are distinguishable by karyometry and PCNA labelling, and their features resemble distinct grades of hepatocellular carcinoma. Histol Histopathol 9:73–83