Histol Histopath (1994) 9: 73-83

Histology and Histopathology

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

M. Zhao[1], N.X. Zhang[2], Z.Y. Du[2], J.A. Laissue[1] and A. Zimmermann[1]

[1]Institute of Pathology of the University of Bern, Bern, Switzerland and

[2]Department of Pathology, Tianjin Medical College, Tianjin, P.R. China

Summary. We have studied the occurrence and specific features of liver cell dysplasia (LCD) in Chinese patients showing liver cirrhosis with or without hepatocellular carcinoma (HCC). Three types of LCD (SLCD, LLCDo, LLCDe) were morphologically defined, and these types were further analyzed using karyometry, estimation of nucleic acid content and density, and PCNA immunostaining. Features found for three types of LCD were compared with those of normal hepatocytes (NLC), simple regenerating hepatocytes (SRLC), and cells of HCCs covering different grades. The results show that 1) karyometry and nucleic acid parameters allow an objective separation of LCD types both from NLC and SRLC; 2) karyometric features of LLCDe are most close to those of highly differentiated HCCs, whereas nuclear size and chromatin composition of SLCD closely reflect those of poorly differentiated HCCs; 3) the frequency of LCD clusters was higher in cirrhotic livers carrying HCC, being about double for all three LCD types; 4) the highest PCNA labelling occurred in the small cell group of LCD (SLCD), still, however, being smaller than that of simple regenerating hepatocytes. Based on these findings it is suggested that, similar to atypical adenomatous hyperplasia, LCDs of distinct morphotypes may represent precursor lesions for HCC, and some cellular forms may mimick cell types known to occur in experimental carcinogenesis.

Key words: Liver cell dysplasia, Cirrhosis, Hepatocellular carcinoma

Introduction

Although cirrhosis of the liver is a major predisposing condition for the development of hepatocellular carcinoma (HCC), characteristic preneoplastic changes have only partly been described in humans (Okuda, 1986). They include liver cell dysplasia (LCD) (Anthony, 1976; Serov and Beketova, 1990), atypical adenomatous hyperplasia (AAH), macroregenerative nodules with bulging activity, and nodule-in-nodule lesions (Anthony et al., 1973; Arakawa et al., 1986; Cohen and Berson, 1986; Roncalli et al., 1986; Karhunen and Penttilä, 1987; Berman and McNeill, 1988; Furuya et al., 1988; Wada et al., 1988; Nakanuma et al., 1989; Paterson et al., 1989; Terada et al., 1989a,b; Crawford, 1990; for reviews see Nakanuma et al., 1990, 1993; Okuda, 1992). LCD was originally detected in the liver of HBsAg-positive Ugandan patients and emphasized as a preneoplastic change (Anthony, 1976), but several other studies arrived at controversial interpretations regarding the preneoplastic nature of LCD (Anthony, 1976; Akagi et al., 1984; Roncalli et al., 1985; Chopra et al., 1987; Kovacs and Elek, 1987; Lefkowitch and Apfelbaum, 1987; Reid et al., 1987; Matturri and Bauer, 1988; Watanabe et al., 1988; Borzio et al., 1991; Hytiroglou et al., 1992). In Western countries, the etiology of liver cirrhosis has not been conclusively shown to be of relevance for the presence of LCD (Ferrel et al., 1992; Theise et al., 1992), but LCD is relatively frequently observed in hepatitis C virus (HCV) infection (Ferrel et al., 1992). The question as to the precancerous significance of LCD as such is complicated by the facts that 1) LCD (and its subtypes) has still not fully been defined, and 2) cellular atypias similar to LCD occur in large liver nodules termed atypical adenomatous hyperplasia (AAH), an entity widely accepted to be a precancerous lesion (Nakanuma et al., 1993). However, immunohistochemical (Roncalli et al., 1985, 1986; Govindarajan et al., 1990) and morphometric (Giannini et al., 1987; Roncalli et al., 1988) studies support the view that foci of dysplastic hepatocytes may represent a cell population prone to neoplastic evolution, and this suggestion has recently

Offprint requests to: Prof. Dr. A. Zimmermann, Pathologisches Institut der Universität, Murtenstrasse 31, CH-3010 Bern, Switzerland

been supported by the finding of DNA ploidy abnormalities in morphologically atypical hepatocytes (Zerbini et al., 1992) and of an abnormal DNA content in LCD (Roncalli et al., 1989).

In order to study LCD in detail, we performed a retrospective light microscopic, karyometric and immunohistochemical investigation on biopsies of cirrhotic livers with or without HCC, but without large nodules of the AAH type. The aim of the present study was fivefold: 1) to morphologically define different types of LCD; 2) to characterize dysplastic hepatocytes of different categories by karyometry, nucleic acid content and nucleic acid density; 3) to assess proliferative activity of dysplastic cells using PCNA staining; 4) to compare these features of LCD with those of normal lobular hepatocytes and of hyperplastic, but not atypical hepatocytes of cirrhotic nodules; and 5) to test as to whether the features of distinct categories of LCD are similar to those which have been described in distinct grades of HCC and for atypical cells in AAH.

Materials and methods

Material for histology

Biopsy and operation specimens of 36 Chinese patients with cirrhosis and HCC and of 66 Chinese patients with cirrhosis but without HCC were retrospectively analyzed. The selection criterion for these cases was the presence of LCD within cirrhotic nodules, and this was identified in a set of 1748 liver biopsies observed between 1957 and 1988 in a Chinese centre (Tables 1, 2). The material analyzed did not contain AAH (Nakanuma et al., 1993).

For conventional light microscopy, tissue samples were fixed in 10% formaldehyde solution, dehydrated and embedded in paraffin. Formalin fixation before embedding was less than 30 hours throughout, which is important for PCNA staining (see below). Sections were stained with haematoxylin and eosin.

Immunohistochemistry

Proliferating cell nuclear antigen (PCNA) immunostaining was performed using the APAAP procedure. Tissue sections were deparaffinized and rehydrated (Tris-NaCl buffer; 0.1% Tris and 1% NaCl, pH 7.4, Merck). The sections were then exposed to 3% BSA (Merck) in Tris-NaCl buffer with 0.1 ml/ml normal horse serum and 0.1% NaN_3 for 45 min at room temperature. Afterwards, the preparations were incubated with a primary mouse monoclonal antibody directed against PCNA (DAKO-PCNA, PC 10) for 1 h. A dilution of 1:50 in Tris-NaCl buffer containing 0.1% NaN_3 was used. Rabbit anti-mouse immunoglobulin (DAKO) at a dilution of 1:30 and APAAP mouse monoclonal antibody (DAKO) at a dilution of 1:50 were applied for 45 min each. Following the respective incubations the sections were rinsed three times in Tris-NaCl buffer. All incubations were performed in a humidified environment at room temperature. The alkaline phosphatase reaction was run for 20 min in New Fuchsin substrate solution, and the reaction was stopped by rinsing the sections in cold tap water. Finally, the sections were counterstained with haematoxylin (Merck) and mounted with Aquadex (Merck). The primary antibody was substituted with normal mouse serum (DAKO) in protein concentration of 0.06 mg/ml Tris-NaCl buffer for negative control sections, and all other steps were performed for these preparations. As a positive control an HCC preparation strongly expressing PCNA was processed in parallel to each incubation step.

Karyometric analyses

5 µm-thick paired paraffin sections were produced and parallely stained (Pearse, 1985) with either haematoxylin-eosin or the Feulgen reagent under strictly controlled conditions. Three parameters, i.e. nuclear area (NA), nucleic acid content (NAC), and the nucleic acid density (NAD) were estimated. NA, as defined here, is the estimated projected area of a nuclear slice represented in a section of 5 µm thickness. NAC is the microphotometrically determined amount of Feulgen-positive material within such a nuclear slice. NAD represents the calculated ratio NAC/NA. A total of 3361 cells (3016 hepatocytes and 345 lymphocytes) were analyzed for the 102 cases (Table 2) using a scanning microspectrophotometer (SMP-03 OPTON, model 1986, Germany) with a monochromatic wavelength of 580 nm, x60 objective, and x16 illuminating lens. Using a standard step length of 1 µm, each nuclear profile was automatically scanned and the tracked measuring positions summed up to NA. Similarly, and using the same step length, NAC per nucleus (in arbitrary units per nuclear profile) was estimated. Lymphocytes represented in the same sections were selected as reference objects.

Assessment of proliferative activity of hepatocytes and tumor cells using PCNA staining

PCNA (cyclin; Mathews et al., 1984; Celis and Celis, 1985; Kurki et al., 1986; Celis et al., 1987; Hall and Woods, 1990) was assessed as previously described (Nakane et al., 1989; Hall et al., 1990; Ottavio et al., 1990; Dervan et al., 1992; Gelb et al., 1992; Jankowski et al., 1992; Kawakita et al., 1992a,b; Kayano et al., 1992; Koukoulis et al., 1992; Linden et al., 1992; McCormick and Hall, 1992; Shivji et al., 1992; Shresta et al., 1992). In normal livers, only very few hepatocyte nuclei show immunohistochemical staining for PCNA, and most positive nuclei are found in the littoral cell population (Koukoulis et al., 1992). In the present study, the PC 10 monoclonal mouse anti-PCNA antibody was used (DAKO-PCNA PC 10) which has recently been shown to stain biopsies more reliably than 19A2 (Gelb et al., 1992). The tissue samples analyzed had been fixed in formalin for less than 30 hours which is important

because both the choice of fixative and the duration of fixation may affect the accessibility of PCNA/cyclin antigen (Bravo and MacDonald-Bravo, 1987; Galand and Degraef, 1989; García et al., 1989; Francis et al., 1992; Gelb et al., 1992).

With the APAAP method used in the present study, nuclei that had reacted with the antibody were stained either pink or brightly red. Thus, even though immunoreactivity was sharply defined (i.e., nuclear staining only), its intensity was not uniform, as previously reported (Koukoulis et al., 1992). This heterogeneity of PCNA staining has to be taken into account when identifying acceptably «positive» nuclei in order to estimate a PCNA labelling index. Pink nuclei may either represent nuclei with incipient PCNA accumulation or daughter nuclei still expressing (diluted) PCNA after having traversed the last cell division cycle, as PCNA has itself a long half-life (Hall et al., 1990; Yu et al., 1992). Therefore, nucleated hepatocytes and HCC cells were considered positive for PCNA only if definite brightly red staining of the nucleus was identified. PCNA labelling was scored using a x40 objective and a x10 eyepiece, and sections were scanned to identify areas that were most evently and heavily labelled, but within these zones areas to be analyzed were randomly chosen. The extent of PCNA positivity was evaluated by determining the percentage of positive nuclei present in nucleated hepatocytes or tumor cells on a single section. This percentage was expressed as the PCNA labelling index.

Statistical analysis

The three parameters, NA, NAC and NAD, obtained with 3361 cells in 8 groups (Table 3) were analyzed with a U test in order to explore karyometric correlations between LCDs and HCCs. 39,912 PCNA-positive hepatocytes were counted in 102 cases of cirrhosis with or without HCC and allocated to four types of hepatocytes according to criteria given below, and an unpaired t-test was employed to analyze the proliferative activity among these four cell types.

Results

Morphological classification of LCD

As a working formulation, dysplastic and regenerating hepatocytes were classified into four groups on the base of morphological features in comparison with normal hepatocytes (NLC):

1) Simple regenerating liver cells/hepatocytes (SRLC).

SRLC were characterized as hepatocytes which were smaller than NLC, but whose nuclear size was apparently normal. The main difference between SRLC and NLC, however, was the typical arrangement of the former: SRLC had a tendency to form clusters within cirrhotic nodules, where they usually occurred in peripheral parts and were then easily visualized due to nuclear crowding, but may form cell cords, as previously described (Weinbren et al., 1985).

2) Large liver cell dysplasia with nuclear hypochromasia (LLCDo; Fig. 1).

These dysplastic hepatocytes were large cells with a large nucleus and one or several prominent nucleoli (Anthony, 1976). Mean nuclear diameter was 14.5 µm. This cell type frequently showed an abundant, eosinophilic or clear cytoplasm. LLCDo usually occurred in clusters and occupied a part of a nodule, but may form entire cirrhotic nodules (Fig. 2).

3) Large liver cell dysplasia with nuclear hyperchromasia (LLCDe; Fig. 1).

LLCDe had some features in common with LLCDo, but it usually exhibited strongly eosinophilic cytoplasm and markedly polymorphous and hyperchromatic nuclei.

Fig. 1. Cirrhotic nodule showing a mixture of the three types of liver cell dysplasia (LCD) as defined in the present study. The top right area of the nodule exhibits a cluster of small cells corresponding to SLCD. The top left area consists of larger cells with nuclear hypochromasia (LLCDo), whereas the bottom area is occupied by large cells with nuclear hyperchromasia (LLCDe). H & E . x 150

Mean nuclear diameter was 13.5 μm, i.e. a little smaller than in LLCDo. In contrast to LLCDo, chromatin structure was coarse, and the nuclear membrane was thick. LLCDe occurred in clusters or formed entire nodules (Fig. 2).

4) Small liver cell dysplasia (SLCD; Watanabe et al., 1988; Fig. 1).

As compared to LLCD, both the cell size and the nuclear size were smaller (mean nuclear diameter: 10.1 μm), but in contrast to SRLC, the cytoplasm was basophilic and the nuclei were hyperchromatic. Cells of SLCD in most instances located in peripheral parts of cirrhotic nodules, where they formed clusters, but they may also form entire nodules (Fig. 2).

SRLC, LLCDo, LLCDe and SLCD were found both in cirrhotic livers with or without HCC, and the four types may coexist within a single cirrhotic nodule. Based on the definitions given above, foci of SRLC, LLCDo, LLCDe and SLCD were found in cirrhotic livers without HCC with a frequency of 33.33%, 13.89%, 2.78% and 22.22% respectively, whereas their frequencies in livers with HCC were 25.76%, 30.30%, 6.06%, and 39.40%, respectively (Tables 1, 2).

Karyometric analysis

The number of cells analyzed for NLC, SRLC, LCD and for HCC are given in Table 3. As seen in Table 4, both LCD and HCC were different from SRLC both with regard to nuclear area and nucleic acid distribution, and this was further broken down for LCD in comparison to SRLC in Table 5. Of the four types of hepatocytes, LLCDo cells had the largest nuclear area (3.5 times larger than SRLC), and also SLCD cells had a nuclear area almost double that of SRLC. SLCD nuclei exhibited the highest nucleic acid density (NAD) among the four cell types, being about twice that of SRLC, whereas the NAD ratio for the two types of large cell dysplasia in comparison to SRLC was close to unity. With respect to NAC, LLCDo, LLCDe and SLCD showed 3.3 to 3.5 times higher values than SRLC.

The results indicate that karyometry and estimation of NAD and NAC allow an objective separation of three LCD types both from NLC and SRLC, whereas SRLC in cirrhotic nodules are identifiable by their spatial

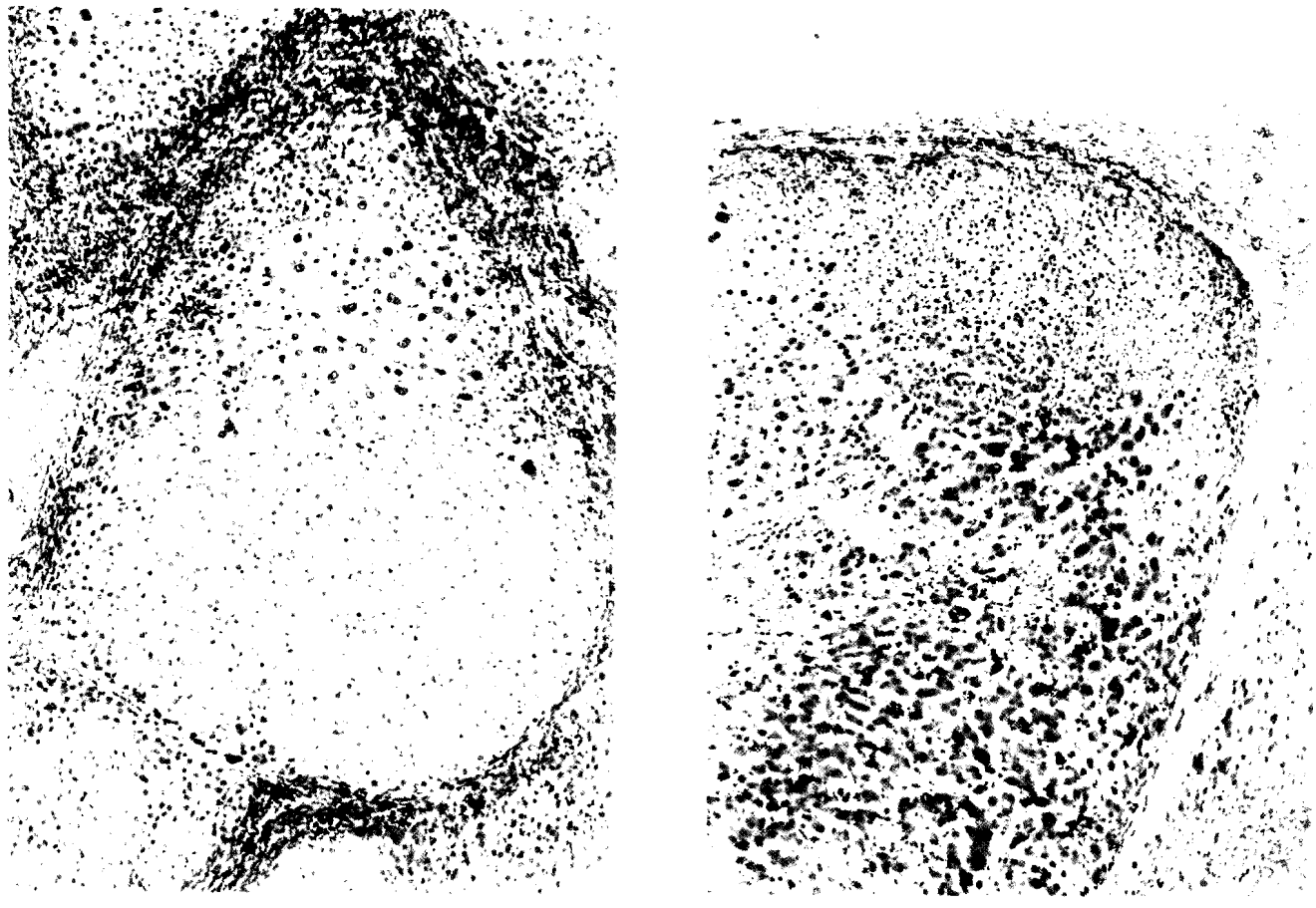

Fig. 2. A. The majority of cells seen in this pear-shaped cirrhotic nodule morphologically correspond to SRLC. A cap of LLCDo is visualized in the top third of the nodule. H & E. x 150. **B.** In contrast to A, most of the cells represented in this nodule are LLCDe, and only a small cluster of SRLC is seen in the right top corner. H & E. x 150

arrangement in clusters.

In a further step, pairs of the eight cell types analyzed were examined for a correlation of karyometric parameters (NA, NAD, NAC) using the U test. Based on a calculated U value of > 1.96, cell types per given pair were different ($p < 0.05$) with respect to either NA, NAD, or NAC, whereas U value < 1.96 means that two partners of a pair had the same or very similar karyometric features. These correlations are listed in Table 6. It is seen that the highest degree of similarity occurs between LLCDe and Highly differentiated HCC (hHCC), followed by the pair, SLCD and poorly differentiated HCC (pHCC). It thus appears that nuclei of pHCCs (as based on the Edmondson-Steiner grading system) exhibit a size and chromatin composition very close to that of the nuclei of the small-cell group of liver cell dysplasia, whereas hHCCs reflect a karyometric pattern close to that of large dysplastic cells with hyperchromasia of the nuclei. These karyometric analyses in an objective way confirm the visual impressions used in conventional grading of HCCs, in that grades 1 and 2 according to Edmondson and Steiner (Okuda and Ishak, 1987) corresponded to those lesions showing a high cytoplasm-to-nucleus ratio and larger nuclei, whereas grades 3 and 4 showed inverse relationships.

PCNA staining

In the present study, 47.6% of HCC nuclei were PCNA-positive. The PCNA labelling indices (LI) of dysplastic, hyperplastic and «normal» hepatocytes

Table 1. Cases of liver cirrhosis or fibrosis associated with HCC.

CASE	HCC										CIRRHOSIS				LCDs		
	Type						Grade				Type				LLCDo	LLCDe	SLCD
1	T			SC			1	2				MI			++	++	+++
2		P						2						FI	+++	++	+
3					F					4	MA				+	++	++
4	T	P						2	3		MA				+++	++	+++
5	T	P		Sc			1	2			MA				+	++	+++
6	T								3	4		MI			+	++	+
7		P	C			Sa			3	4		MI					+
8			C					2			MA				+	++	+
9						Sa				4				FI	+		
10	T							2				MI			+	++	+
11	T			Sc			1	2				MI					++
12		P						2	3				MX			++	
13		P							3					FI			++
14			C					2	3					FI			+
15	T								3	4				FI		++	+
16	T								3			MI			+++	++	++
17	T							2			MA				+++	++	++
18	T			Sc					3	4		MI					+
19	T									4		MI					++
20			C					2	3			MI					++
21				Sc	F					4		MI				++	++
22	T								3			MI				+	+++
23	T		C					2	3		MA					++	+++
24	T							2				MI				++	+++
25	T							2				MI			+	++	+
26	T							2				MI					++
27	T							2			MA						++
28	T							2	3			MI					++
29		P						2	3					FI		+	+
30			C			Sa				4		MI				+	+++
31	T									4	MA						++
32		P				Sa				4	MA					+	++
33	T								3	4	MA						++
34	T							2	3			MI				+	
35	T		C					2	3		MA				+++	+++	
36	T								3			MI			+		
TOTAL	23	8	7	5	2	4	3	20	18	12	11	18	1	6	14	22	31
%	63.87	22.22	19.49	13.89	5.56	11.11	8.33	55.56	50.00	33.33	30.56	50.00	2.78	16.67	38.89	61.11	86.11

HCC type (Nakashima and Kojiro, 1987): T, trabecular (sinusoidal) type; P, pseudoglandular (acinar) type; C, Compact (solid) type; Sc, scirrhous (sclerosing) type; f, fatty HCC. HCC grade: Grades 1-4 according to Edmonson and Steiner (1954). Cirrhosis/fibrosis: MA, macronodular; MI, micronodular; MX, mixed nodular; FI, liver fibrosis. LCDs: liver cell dysplasia (see text): +, LCD single cell or in cluster; ++, LCD cells in cluster or ocupying part of cirrhotic nodule; +++, LCD cells form cirrhotic nodule.

Table 2. Cases of liver cirrhosis or fibrosis without HCC.

CASE	TYPE				LLCDo	LLCDe	SLCD
1	MA					+	++
2			MX				++
3		MI			+	++	
4				FI			+++
5				FI			+++
6				FI			+++
7				FI			+++
8		MI					+++
9				FI	+	++	+++
10			MX		+	++	+
11		MI			++	+++	+++
12			MX		+++	+	
13	MA				+++	+++	+
14	MA				++	++	+
15	MA				+++	+	+++
16				FI			+
17		MI			++	+	+
18	MA					+++	
19	MA						+++
20			MX				+++
21	MA				+++	++	
22		MI					++
23		MI					++
24		MI					++
25	MA				+++	++	+++
26			MX				++
27				FI			+
28				FI	+++		+++
29	MA						++
30				FI	++	+	
31	MA				+++	++	+++
32			MX			+	+++
33	MA				+++	++	++
34		MI			+		+
35		MI			++		+++
36		MI			++	+	+++
37	MA						++
38		MI			+		+
39	MA				++		
40				FI			++
41		MI				++	+
42		MI			+++		+
43		MI			+++	+++	+++
44		MI			++		+++
45	MA					+++	+++
46		MI			++		
47		MI			++	+	+++
48		MI			+++		
49		MI			+++	+	+++
50	MA				+++		+++
51	MA				+		++
52			MX		+++	+	+
53	MA				++		++
54		MI			++		+
55				FI	+		
56				FI	++	+	+++
57	MA				++		
58			MX		+++		+++
59		MI			+++	+	+++
60			MX		+++	+	
61		MI			++	+	+++
62				FI	+++		+
63	MA						++
64				FI	++		+++
65	MA				+++	++	+
66		MI			++	+	
TOTAL	20	23	9	14	43	30	54

Abbreviations: See Table 1.

(NLC) occurring in liver cirrhosis associated with HCC are shown in Figure 3. 6.5% of NLC were PCNA-positive, which is higher than the values obtained for normal liver tissue using ^{3}H-thymidine labelling (Grisham, 1962), a phenomenon discussed for formalin-fixed tissue in a previous report (Galand and Degraef, 1989), and attributed to differences in geometrical constraints affecting the detection of signals and to the presence of cyclin in G1 and G2 cells (Thaete et al., 1989). It is interesting to note that the highest LI was found in the group of intranodal hyperplastic cells without nuclear atypia (SRLC), being 8.8 times higher than that of NLC. Among the three groups of liver cell dysplasia, the highest LI was found for SLCD (17.7%), whereas LIs of LLCDo and LLCDe were close to each other (8.9 and 9.9, respectively) and significantly different from SLCD, but not from NLC. Figure 3 further illustrates that the distribution of PCNA-LIs for the different cell groups occurring in cirrhosis not associated with HCC were basically similar, but with

Table 3. Cell types analyzed for karyometric analysis.

TYPE OF LIVER DISEASE/CONTROL	NUMBER OF CASES	CELL TYPE ANALYZED	NUMBER OF CELLS ANALYZED (TOTAL PER DISEASE/ CONTROL GROUP)
Cirrhosis without LCD	20	SRLC	492
Cirrhosis with LCD	46	LLCDo	477
		LLCDe	438
		SLCD	1010
hHCC	12	HCC	201
mHCC	12	HCC	198
pHCC	12	HCC	200
Control		Lymphocyte	345

LCD: liver cell dysplasia; LLCDo: large liver cell dysplasia with nuclear hypochromasia; LLCDe: large liver cell dysplasia with nuclear hyperchromasia; SLCD: small liver cell dysplasia; SRLC: simple regenerating hepatocyte; HCC: hepatocellular carcinoma; hHCC: HCC, highly differentiated; mHCC: HCC, moderately differentiated; pHCC: HCC, poorly differentiated.

Table 4. Karyometric features of dysplastic vs. hyperplastic and neoplastic hepatocytes.

CELL TYPE	NA	NAC	NAD
SRH	46.79±10.21	25.49±6.81	0.54±0.07
LLCDo	164.73±58.62	88.48±34.31	0.53±0.07
LLCDe	142.07±45.51	86.48±27.94	0.61±0.11
SLCD	79.25±36.39	85.18±39.76	1.07±0.12
hHCC	145.74±109.98	91.51±70.95	0.62±0.83
mHCC	79.48±32.06	57.90±26.60	0.72±0.11
pHCC	76.70±35.55	85.59±45.09	1.10±0.11
Lympocytes	20.15±4.01	15.17±3.09	0.76±0.12

Abbreviations for cell types are as in Table 3. NA: nuclear area (μm^2); NAC: nucleic acid content (arbitrary units); NAD: nucleic acid density (ratio NAC/NA). Mean±SD.

Table 5. Comparison of LCD with SRLC.

TYPE OF LCD	NA/SRLC (μm^2)	ND/SRLC (μm)	NAC/SRLC	NAD/SRLC
LLCDo	164.7/46.8≈3.5	14.5/7.7≈1.9	88.48/25.5≈3.5	0.54/0.55≈0.98
LLCDe	142.1/46.8≈3.0	13.5/7.7≈1.8	86.88/25.5≈3.4	0.62/0.55≈1.13
SLCD	79.3/46.8≈1.7	10.1/7.7≈1.3	85.19/25.5≈3.3	1.04/0.55≈1.89

Table 6. Correlation matrix (NA, NAD, NAC) for eight groups of non-neoplastic and neoplastic hepatocytes.

CELL GROUP	NA	NAC	NAD
SRLC LLCDo	43.302	39.350	01.659*
SRLC LLCe	42.859	44.813	11.659
SRLC SLCD	19.750	34.121	83.023
SRLC hHCC	12.732	13.167	11.427
SRLC mHCC	14.059	16.920	19.249
SRLC pHCC	11.700	18.761	55.618
SRLC Lymphocytes	52.358	29.549	27.988
LLCDo LLCDe	06.556	00.774*	12.766
LLCDo SLCD	27.444	01.412*	83.279
LLCDo hHCC	02.311	00.578	12.481
LLCDo mHCC	24.210	12.440	20.011
LLCDo pHCC	23.931	00.811	56.145
LLCDo Lymphocytes	53.686	46.404	28.786
LLCDe SLCD	23.373	00.779*	59.373
LLCDe hHCC	00.455*	00.893	00.376*
LLCDe mHCC	19.871	12.523	10.065
LLCDe pHCC	19.662	00.373*	44.354
LLCDe Lymphocytes	55.783	53.300	16.177
SLCD hHCC	08.394	01.195*	56.556
SLCD mHCC	00.670*	10.699	35.152
SLCD pHCC	00.872	00.133*	02.488*
SLCD Lymphocytes	37.170	40.463	35.145

Abbreviations are as in Table 3. Based on the U test (see Materials and methods), U values > 1.96 mean that respective pairs of cells are not different or very similar to NA NAD, or NAC. These situations ($p > 0.05$) are marked with and asterisk.

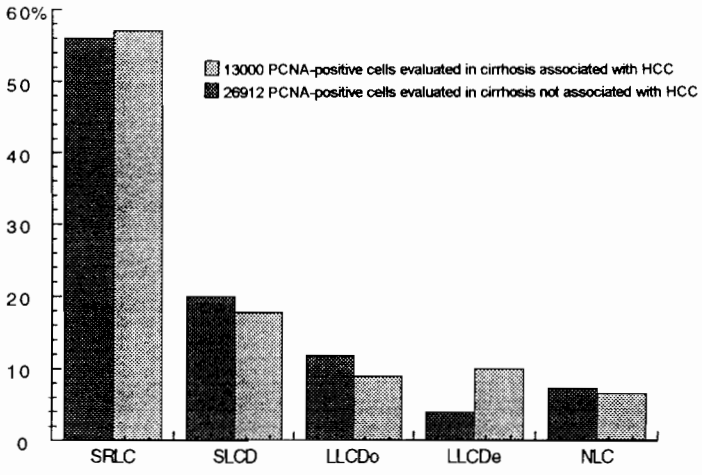

Fig. 3. PCNA labelling indices for normal, hyperplastic and dysplastic hepatocytes.

one exception. With 3.7% the LI of LLCDe was 2.6 times lower than that of LLCDe in the group with HCC, and was statistically different from both SLCD and LLCDo. LI of NLC in the cirrhosis only group was close to the respective value found in the group associated with HCC.

Discussion

Apart from overt HCC, cellular and structural atypias occur in a group of hepatocellular lesions currently discussed with respect to their precancerous significance, i.e. LCD (Anthony, 1976) and AAH, MRN type II (Nakanuma et al., 1993). The preneoplastic potential of LCD has been questioned (Watanabe et al., 1988), whereas that of AAH appears to become established (Nakanuma et al., 1993). AAH is accepted to represent large, i.e. grossly visible lesions (Nakanuma et al., 1990; Theise et al., 1992). In contrast, LCD frequently occurs in small cirrhotic nodules not visualized by imaging, and its evolution is not really known, even though small dysplastic foci may coexist with HCC in other parts of the liver (Nieburgs et al., 1965; Furuya et al., 1988; Wada et al., 1988; Nakanuma et al., 1990).

Based on a combined approach we have shown that the visual impression of small regenerating hepatocytes (SRLC) being different even from the category of LCD showing the smallest cells (SLCD) is supported by karyometry, in that SLCD cells exhibit a nuclear area almost double that of SRLC, their nuclei show the highest NAD among the four cell types analyzed, and their NAD is about twice that of SRLC nuclei. SLCD cells were observed in 22% of cirrhotic livers without HCC, but were more frequently noted in cirrhotic livers harbouring HCC (39%). As the overall features of cirrhosis were fairly homogeneous for the two groups with or without HCC, we do not think that this difference solely reflects a sampling effect. In comparison with both SRLC and SLCD, large dysplastic cells (LLCDo and LLCDe) are more easily detectable. Of the four types of non-normal hepatocytes, LLCDo cells showed the largest NA (3.5 times larger than that of SRLC). LLCDo cells were noted in 13.8% of cirrhotic livers without HCC, but similar to SCLD, they were more frequently encountered in cirrhosis associated with HCC, and the difference was more important (30.3% vs. 13.8%). This observation is supported by previously published data from South Africa (Paterson et al., 1989). In contrast to LLCDo, LLCDe cells appear to be much rarer (3 to 6%), even though their frequency is also higher in cases with HCC.

These findings indicate that karyometry allows an objective separation of LCD from NLC and SRLC, which has previously been suggested (Henmi et al., 1985; Giannini et al., 1987; Matturri and Bauer, 1988; Nagato et al., 1991). In contrast to a former study, however (Henmi et al., 1985), we do not agree that all LCDs show marked increases in DNA content. In fact, differences may be related to geometrical constraints (small vs. large nuclear volumes) and, therefore, to variable chromatin packing rather than to real differences in overall DNA content. This is illustrated by a particularly high NAD in SCLD in comparison with LLCDo. Theoretically, one might assume that sets of atypical cells deviating from the normal counterpart should form a more or less continuous spectrum of anomalous phenotypes, ranging from a normal type to the most severe atypia. Obviously, for LCD this seems not to be the case. The reason for the occurrence of relatively few morphotypes is not clear, but it was of interest to analyze this phenomenon in relation to cellular features of overt HCCs.

The present correlative karyometric analysis showed that the highest degree of similarity occurred between LLCDe and HCC of high differentiation (hHCC), followed by the pair, SLCD and HCC of low differentiation (pHCC), indicating that nuclei of hHCCs exhibit a size and chromatin composition very close to that of the nuclei of large LCD with hyperchromatic nuclei, whereas pHCCs reflect a karyometric pattern very similar to that of the small-cell group of LCD. This relationship is of interest with respect to experimental hepatocarcinogenesis, where preneoplastic foci consisting of large and glycogen-rich hepatocytes have been described (Bannasch, 1978; Schauer and Kunze, 1978; Evarts et al., 1990). The transformation into HCC appears to take place through progressive loss of glycogen, and a population of smaller, basophilic cells ensues (Bannasch, 1968; Forget and Daoust, 1970; Hsia et al., 1992), associated with increased proliferation activity (Schauer and Kunze, 1978). Results of this type can only be compared with the human situation with caution (National Academy of Sciences, 1980), but basophilic and proliferative cells seen in animal experimentation resemble SLCD, whereas LLCDo cells with their clear cytoplasm may correspond to the large cells observed within glycogen storage islands, and the eosinophilic LLCDe cells to so-called acidophilic cells, occurring in experimental situations (Bannasch, 1978), the latter representing a transition form within the sequence of glycogen loss. In contrast it has been noted that the widespread nature of large cell dysplasia may weaken its status as a premalignant lesion, but may rather represent a marker of chronic liver injury (Cohen and Berson, 1986; Theise et al., 1992). Based on the differences in frequency between cases with and without HCC this view should be revised, and we were interested to test as to whether LCD types are, therefore, different with respect to proliferative activity.

SLCD cells exhibited the highest PCNA labelling, but it was still smaller than that of hyperplastic cells (SRLC) which showed a PCNA LI 8.8 times higher than that of NLC. It has been shown that DNA synthesis potency in noncancerous cirrhotic tissue from patients with liver cirrhosis complicated by HCC is significantly higher than that in cirrhotic livers not associated with HCC, suggesting that HCC may develop in cirrhotic

patients with high DNA synthesis rate (Tarao et al., 1991, 1992). Moreover, flow cytometrical analyses have shown that DNA anomalies are more frequent in LCD of high grade than in low grade (Thomas et al., 1992). SLCD with its consistently higher PCNA labelling was more frequently observed in cirrhosis associated with HCC in the present study. In contrast, labelling of LLCDo and LLCDe cells was lower, (8.9% and 9.9%, respectively), significantly different from SCLD, but not from NLC. As has been emphasized above, PCNA labelling may overestimate the proliferative activity of cells (Grisham, 1962; Galand and Degraef, 1989). However, based on the fact that fixation of samples was homogeneous throughout (Koukoulis et al., 1992) we assume that the morphological and proliferative patterns seen with LCD may in some way mimick the events occurring in experimental carcinogenesis, and that LCD, similar to AAH, may therefore represent a preneoplastic compartment of hepatocytes.

What is the relationship between LCD and AAH? In a recent study, the mean values of PCNA labelling of large regenerative nodules, ordinary adenomatous hyperplasia, atypical adenomatous hyperplasia, and adenomatous hyperplasia containing cancerous foci were 1.9, 4.1, 6.4, and 9.6%, respectively (Eguchi et al., 1992), suggesting an increase of proliferative activity with increasing atypia and switching to overt malignancy. Should this reflect a systematic effect within the sequence of events leading to neoplasia (Tsuda et al., 1988; Kondo et al., 1990; Matsuno et al., 1990; Takayama et al., 1990; Sakamoto et al., 1991; Terada and Nakanuma, 1992), then LLCD with its lower labelling may represent a more proximal lesion, whereas SCLD with its higher labelling may represent a lesion more close to the pattern seen in AAH or borderline lesions.

In conclusion, subtypes of LCD occurring in liver cirrhosis are lesions with distinct morphological and karyometric features. They may share karyometric characteristics with distinct grades of HCC, and exhibit different patterns of proliferative activity; SLCD showing the highest activity as based on PCNA labelling. Moreover, subtypes of LCD may reflect some stages in carcinogenesis and appear to show similarities to lesions known to occur in the spectrum of adenomatous hyperplasia.

Acknowledgements. The technical help of Mary Economou is gratefully acknowledged. We thank Erna Müller for her expertise in writing the manuscript.

References

Akagi G., Furuya K., Kanamura A., Chihara T. and Otsuka H. (1984). Liver cell dysplasia and hepatitis B surface antigen carriers in liver cirrhosis and hepatocellular carcinoma. Cancer 54, 315-318.

Anthony P.P. (1976). Precursor lesions for liver cancer in humans. Cancer Res. 36, 2579-2583.

Anthony P.P., Vogel C.L. and Barker L.F. (1973). Liver cell dysplasia: A prema-lignant condition. J. Clin. Pathol. 26, 217-223.

Arakawa M., Kage M., Sugihara S., Nakashima T., Suenaga M. and Okuda K. (1986). Emergence of malignant lesions within an adenomatous hyperplastic nodule in a cirrhotic liver: observation in five cases. Gastroenterology 91, 198-208.

Bannasch P. (1968). The cytoplasm of hepatocytes during carcinogenesis. Recent results of cancer research. Vol. 19. Springer. Berlin.

Bannasch P. (1978). Cellular and subcellular pathology of liver carcinogenesis. In: Primary liver tumours. Remmer H., Bannasch P., Bolt H.M. and Popper H. (eds). MTP Press Limited, Lancaster. England. pp 87-111.

Berman J.J. and McNeill R.E. (1988). Cirrhosis with atypia: A potential pitfall in the interpretation of liver aspirates. Acta Cytol. 32, 11-14.

Borzio M., Bruno S., Roncalli M., Mels G.C., Ramella G., Borzio F., Leandro G. and Podda M. (1991). Liver cell dysplasia and risk of hepato-cellular carcinoma: a preliminary report. Brit. Med. J. 302, 1312.

Bravo R. and Macdonald-Bravo H. (1987). Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: Association with DNA replication sites. J. Cell Biol. 105, 1549-1554.

Celis J.E. and Celis A. (1985). Cell cycle-dependent variations in the distribution of the nuclear protein cyclin/proliferating cell nuclear antigen in cultured cells: subdivision of S phase. Proc. Natl. Acad. Sci. USA 82, 3262-3266.

Celis J.E., Madsen P., Celis A., Nielsen H.V. and Gesser B. (1987). Cyclin (PCNA, auxiliary protein of DNA polymerase delta) is a central component of the pathway(s) leading to DNA replication. FEBS Lett. 220, 1-7.

Chopra P., Vijayraghvan M. and Nayak N.C. (1987). Significance of liver cell dysplasia in cirrhosis and hepatocellular carcinoma. Ind. J. Med. Res. 86P, 382-390.

Cohen C. and Berson S.D. (1986). Liver cell dysplasia in normal cirrhotic and hepatocellular carcinoma patients. Cancer 57, 1535-1538.

Crawford J. (1990). Pathologic assessment of liver cell dysplasia and benign liver tumors: differentiation from malignant tumors. Semin. Diagn. Pathol. 7, 115-128.

Dervan P.A., Magee H.M., Buckley C. and Carney D.N. (1992). Proliferating cell nuclear antigen counts in formalin-fixed paraffin-embedded tissue correlate with Ki-67 in fresh tissue. Am. J. Clin. Pathol. 97 (suppl. 1), S21-S28.

Eguchi A., Nakashima O., Okudaira S., Sugihara S. and Kojiro M. (1992). Adenomatous hyperplasia in the vicinity of small hepato-cellular carcinoma. Hepatology 15, 843-848.

Edmonson H.A. and Steiner P.E. (1954). Primary carcinoma of the liver. A study of 100 cases among 48900 necropsies. Cancer 7, 462-503.

Evarts R.P., Nakatsukasa H., Marsden E.R., Hsia C.C., Dunsford H.A. and Thorgeirrson S. (1990). Cellular and molecular changes in the early stages of chemical hepatocarcinogenesis in the rat. Cancer Res. 5, 3439-3444.

Ferrell L., Wright T., Lake J., Roberts J. and Ascher N. (1992). Incidence and diagnostic features of macroregenerative nodules vs. small hepatocellular carcinoma in cirrhotic livers. Hepatology 16, 1372-1381.

Forget A. and Daoust R. (1970). Histochemical study on rat liver glycogen during DAB carcinogenesis. Int. J. Cancer 5, 404-410.

Francis S.E., Holt C.M., Gadson P.A. and Angelini G.D. (1992). Another

cautionary note on the use of PCNA. J. Pathol. 166, 418.

Furuya K., Nakamura M., Yamamoto Y., Togei K. and Otsuka H. (1988). Macroregenerative nodules of the liver: a clinicopathologic study of 345 autopsy cases of chronic liver disease. Cancer 61, 99-105.

Galand P. and Degraef C. (1989). Cyclin/PCNA immunostaining as an alternative to tritiated thymidine pulse labelling for marking S phase cells in paraffin sections from animal and human tissues. Cell Tissue Kinet. 22, 383-392.

Garcia R.L., Coltrera M.D. and Gown A.M. (1989). Analysis of proliferative grade using anti-PCNA/cyclin monoclonal antibodies in fixed, embedded tissues. Am. J. Pathol. 135, 733-739.

Gelb A.B., Kamel O.W., LeBrun D.P. and Warnke R.A. (1992). Estimation of tumor growth fractions in archival formalin-fixed, paraffin-embedded tissues using two anti-PCNA/cyclin monoclonal antibodies. Factors affecting reactivity. Am. J. Pathol. 141, 1453-1458.

Giannini A., Zampi G., Bartoloni F. and Omer S. (1987). Morphological precursors of hepatocellular carcinoma: a morphometric analysis. Hepatogastroenterology 34, 95-97.

Govindarajan S., Conrad A., Lim B., ValinluckB., Kim A.M. and Schmid P. (1990). Study of preneoplastic changes of liver cells by immunohistochemical and molecular hybridization techniques. Arch. Pathol. Lab. Med. 114, 1042-1045.

Grisham J.W. (1962). Morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver: autoradiography with ^{3}H thymidine. Cancer Res. 22, 140-146.

Hall P.A. and Woods A.L. (1990). Immunohistochemical markers of cell proliferation. Cell Tissue Kinet. 23, 505-522.

Hall P.A., Levison D.A., Woods A.L., Yu C.C., Kellock D.B., Watkins J.A., Barnes D.M., Gillet C.E., Camplejohn R., Dover R., Waseem N.H. and Lane D.P. (1990). Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: An index of cell proliferation with evidence of deregulated expression in some neoplasms. J. Pathol. 162, 285-294.

Henmi A., Uchida T. and Shikata T. (1985). Karyometric analysis of liver cell dysplasia and hepatocellular carcinoma. Evidence against precancerous nature of liver cell dysplasia. Cancer 55, 2594-2599.

Hsia C.C., Evarts R.P., Nakatsukasa H., Marsden E.R. and Thorgeirsson S. (1992). Occurrence of oval-type cells in hepatitis B virus-associated human hepatocarcinogenesis. Hepatology 16, 1327-1333.

Hytiroglou P., Theise N., Schwartz M., Miller C. and Thung S.N. (1992). Macroregenerative nodules, liver cell dysplasia and hepatocellular carcinoma in adult cirrhotic liver transplants. Lab. Invest. 66. 98A.

Jankowski J., McMenemin R., Hopwood D. and Wormsley K.G. (1992). Proliferating cell nuclear antigen in oesophageal diseases: correlation with transforming growth factor alpha expression. Gut 33, 587-591.

Karhunen P.J. and Penttilä A. (1987). Preneoplastic lesions of human liver. Hepatogastroenterology 34, 10-15.

Kawakita N., Seki S., Sakaguchi H., Yanai A., Kuroki T., Mizoguchi Y., Kobayashi K. and Monna T. (1992a). Analysis of proliferating hepatocytes using a monoclonal antibody against proliferating cell nuclear antigen/cyclin in embedded tissues from various liver diseases fixed in formaldehyde. Am. J. Pathol. 140, 513-520.

Kawakita N., Seki S., Yanai A., Sakaguchi H., Kuroki T., Mizoguchi Y., Kobayashi K. and Monna T. (1992b). Immunocytochemical identification of proliferative hepatocytes using monoclonal antibody to proliferating cell nuclear antigen (PCNA/cyclin). Comparison with immunocytochemical staining for DNA polymerase-alpha. Am. J. Clin. Pathol. 97 (Suppl. 1). S14-S20.

Kayano K., Yasunaga M., Kubota M., Takenaga K., Mori K., Yamashita A., Kubo Y., Sakaida I., Okita K, and Sanuki K. (1992). Detection of proliferating hepatocytes by immunohistochemical staining for proliferating cell nuclear antigen (PCNA) in patients with acute hepatic failure. Liver 12, 132-136.

Kondo F., Ebara M., Sugiura N., Wada K., Kita K., Hirooka N., Nagato Y., Kondo Y., Ohto M. and Okuda K. (1990). Histological features and clinical course of large regenerative nodules: evaluation of their precancerous potentiality. Hepatology 12, 592-598.

Koukoulis G., Rayner A., Tan K.C., Williams R. and Portmann B. (1992). Immunolocalization of regenerating cells after submassive liver necrosis using PCNA staining. J. Pathol. 166, 359-368.

Kovacs L. and Elek G. (1987). Large cell dysplasia of hepatocytes, is it a premalignant condition? Acta Morphol. Hung. 35, 47-57.

Kurki P., Vanderlaan M., Dolbeare F., Gray J. and Tan E.M. (1986). Expression of proliferating cell nuclear antigen (PCNA/cyclin) during the cell cycle. Exp. Cell Res. 166, 209-219.

Lefkowitch J.H. and Apfelbaum T.F. (1987). Liver cell dysplasia and hepatocellular carcinoma in non-A, non-B hepatitis. Arch. Pathol. Lab. Med. 111, 170-173.

Linden M.D., Torres F.X., Kubus J. and Zarbo R.J. (1992). Clinical application of morphologic and immunocytochemical assessments of cell proliferation. Am. J. Clin. Pathol. 97 (Suppl. 1), S4-S13.

Mathews M.B., Bernstein R.M., Franza B.R. and Garrels J.I. (1984). Identity of the proliferating cell nuclear antigen and cyclin. Nature 303, 374-376.

Matsuno Y., Hirohashi S., Furuya S., Sakamoto M., Mukai K. and Shimosato Y. (1990). Heterogeneity of proliferative activity in nodule-in-nodule lesions of small hepatocellular carcinoma. Jpn. J. Cancer Res. 81, 1137-1140.

Matturri L. and Bauer D. (1988). Morphometric characteristics of hepatocellular dysplasia. Anal. Quant. Cytol. Histol. 10, 339-342.

McCormick D. and Hall P.A. (1992). The complexities of proliferating cell nuclear antigen. Histopathology 21, 591-594.

Nagato Y., Dondo F., Kondo Y. and Ohto M. (1991). Histological and morphometrical indicators for a biopsy diagnosis of well-differentiated hepatocellular carcinoma. Hepatology 14, 473-478.

Nakane P.K., Moriuci T., Koji T., Taniguchi Y., Izumi S. and Hui L. (1989). Proliferating cell nuclear antigen (PCNA/cyclin): review and some new findings. Acta Histochem. Cytochem. 22, 105-116.

Nakanuma Y., Kanel G.C., Doishita K., Ohta G. and Peters R.L. (1989). Risk lesions in cirrhosis and development of hepatocellular carcinoma: an autopsy study. Biomed. Pharmacother. 43, 127-133.

Nakanuma Y., Terada T., Terasaki S., Ueda K., Nonomura A., Kawahara E. and Matsui O. (1990). «Atypical adeno-matous hyperplasia» in liver cirrhosis: low-grade hepatocellular carcinoma or borderline lesions? Histopathology 17, 27-35.

Nakanuma Y., Terada T., Ueda K., Terasaki S., Nonomura A. and Matsui O. (1993). Adenomatous hyperplasia of the liver as a precancerous lesions. Liver 13, 1-9.

Nakashima T. and Kojiro M. (1987). Hepatocellular carcinoma. An atlas of its pathology. Springer. Tokyo.

National Academy of Sicences (1980). Histologic typing of liver tumours of the rat. J. Ntl. Cancer Inst. 64, 177-206.

Nieburgs H.E., Parets A.D., Perntz V. and Boudreau C. (1965). Cellular changes in liver tissue adjacent and distant to malignant tumors. Arch. Pathol. 80, 262-272.

Okuda K. (1986). What is the precancerous lesion for hepatocellular carcinoma in man? Gastroenterol. Hepatol. 1, 79-85.

Okuda K. (1992). Hepatocellular carcinoma: recent progress. Hepatology 15, 948-963.

Okuda K. and Ishak K.G. (1987). Neoplasms of the liver. Berlin. Springer. pp 177-187.

Ottavio L., Chang C.D., Rizzo M.G., Travalli S., Casadevall C. and Baserga R. (1990). Importance of introns in the growth regulation of mRNA levels of the proliferating nuclear cell antigen. Mol. Cell Biol. 10, 303-309.

Paterson A.C., Kew M.C., Dusheiko G.M. and Isaacson C. (1989). Liver cell dysplasia accompanying hepatocellular carcinoma in Southern Africa. Differences between urban and rural populations. J. Hepatol. 8, 241-248.

Pearse A.G.E. (1985). Histochemistry: Theoretical and applied. Analytical technology. Vol. 2. Churchill-Livingsyone.

Reid C.L., Wiener G.J., Cox D.W., Richter J.E. and Geisinger K.R. (1987). Diffuse hepatocellular dysplasia and carcinoma associated with the Mmalton variant of alpha 1-antitrypsin. Gastroenterology 93, 181-187.

Roncalli M., Borzio M., De Biagi G., Servida E., Cantaboni A., Sironi M. and Taccagni G.L. (1985). Liver cell dysplasia and hepatocellular carcinoma: A histological and immunohistochemical study. Histopathology 9, 209-211.

Roncalli M., Borzio M., De Biagi G., Ferrari A.R., Macchi R., Tombesi V.M. and Servida E. (1986). Liver cell dysplasia in cirrhosis: A serologic and immunohistochemical study. Cancer 57, 1515-1521.

Roncalli M., Borzio M., Tombesi M.V., Ferrari A. and Servida E. (1988). A morphometric study of liver cell dysplasia. Hum. Pathol. 19, 471-474.

Roncalli M., Borzio M., Brando B., Colloredo G. and Servida E. (1989). Abnormal DNA content in liver cell dysplasia: A flow cytometric study. Int. J. Cancer 44, 204-207.

Sakamoto M., Hirohashi S. and Shimosato Y. (1991). Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. Hum. Pathol. 22, 172-178.

Schauer A. and Kunze E. (1978). Histogenesis of primary liver tumors. In: Primary liver tumors. Remmer H., Bannasch P., Bolt H.M. and Popper H. (eds). MTP Press Limited. Lancaster. England. pp 113-123.

Serov V.V. and Beketova T.P. (1990). The morphological characteristics of hepatocyte dysplasia. Arkh. Patol. 52, 36-42.

Shivji M.K.K., Kenny M.K. and Wood R.D. (1992). Proliferating cell nuclear antigen is required for DNA excision repair. Cell 69, 367-374.

Shresta P., Yamada K., Wada T., Maeda S., Watatani M., Yasutomi M., Takagi H. and Mori M. (1992). Proliferating cell nuclear antigen in breast lesions: correlation of c-erbB-2 oncoprotein and EGF receptor and its clinicopathological significance in breast cancer. Virchows Arch. (A) 421, 193-202.

Takayama T., Makuuchi M., Hirohashi S., Sakamoto M., Okazaki N., Takayasu K., Kosuge T., Motoo Y., Yamazaki S. and Hasegawa H. (1990). Malignant transformation of adenomatous hyperplasia to hepatocellular carcinoma. Lancet 336, 1150-1153.

Tarao K., Shimizu A., Harada M., Ohkawa S., Okamoto N., Kuni Y., Ito Y., Tamai S., Iimori K., Sugisama I., Takemiya S., Okamoto T., Inoue T. and Kanisawa M. (1991). In vitro uptake of bromodeoxyuridine by human hepatocellular carcinoma and its relation to histopathologic findings and biologic behavior. Cancer 68, 1789-1794.

Tarao K., Shimizu A., Ohkawa S., Harada M., Ito Y., Tamai S.,Kuni Y., Okamoto N., Inoue T. and Kanisawa M. (1992). Development of hepato-cellular carcinoma associated with increases in DNA synthesis in the surrounding cirrhosis. Gastroenterology 103, 595-600.

Terada T., Hoso M., Saito K., Kurumaya H. and Nakanuma Y. (1989a). Iron negative foci in siderotic macroregenerative nodules in human cirrhotic liver: A marker of preneoplasia or neoplasia. Arch. Pathol. Lab. Med. 113, 141-148.

Terada T., Nakanuma Y., Hoso M., Saito K., Sasaki M. and Nonomura A. (1989b). Fatty macroregenerative nodule in non-steatotic liver cirrhosis. Virchows Arch. (A) 415, 131-136.

Terada T. and Nakanuma Y. (1992). Cell proliferative activity in adenomatous hyperplasia of the liver and small hepatocellular carcinoma. An immunohistochemical study demonstrating proliferating cell nuclear antigen. Cancer 70, 591-598.

Thaete L.G., Ahnen D.J. and Malkinson A.M. (1989). Proliferating cell nuclear antigen (PCNA/cyclin) immunohistochemistry as a labeling index in mouse lung tissues. Cell Tissue Res. 256, 167-170.

Theise N.D., Schwartz M., Miller C. and Thung S.N. (1992). Macroregenerative nodules and hepatocellular carcinoma in forty-four sequential adult liver explants with cirrhosis. Hepatology. 16, 949-955.

Thomas R.M., Berman J.J., Yetter R.A., Moore W. and Hutchins G.M. (1992). Liver cell dysplasia: A DNA aneuploid lesion with distinct morphologic features. Hum. Pathol. 23, 496-503.

Tsuda H., Hirohashi S., Shimamoto Y., Terada M. and Hasegawa H. (1988). Clonal origin of atypical adenomatous hyperplasia and clonal identity with hepatocellular carcinoma. Gastroenterology 95, 1664-1666.

Wada K., Kondo Y. and Kondo F. (1988). Large regenerative nodules and dysplastic nodules in cirrhotic livers: a histopathologic study. Hepatology 8, 1684-1688.

Watanabe S., Okita S., Harada T., Kodama T., Numa Y., Takemoto T. and Takahashi T. (1988). Morphologic studies of the liver cell dysplasia. Cancer 51, 2197-2205.

Weinbren K., Hadjis N.S. and Blumgart L.H. (1985). Structural aspects of the liver in patients with biliary disease and portal hypertension. J. Clin. Pathol. 38, 1013-1020.

Yu C.C.W., Hall P.A., Fletcher C.D.M., Camplejohn R.S., Waseem N.H., Lane D.P. and Levison D.A. (1992). Haemangio-pericytomas: the prognostic value of immunohistochemical staining with a monoclonal antibody to proliferative cell nuclear antigen (PCNA). Histopathology 19, 29-33.

Zerbini C., Weinberg D.S., Hollister K.A. and Perez-Atayde A.R. (1992). DNA ploidy abnormalities in the liver of children with hereditary tyrosinemia type I. Correlation with histopathologic features. Am. J. Pathol. 140, 1111-1119.

Accepted September 3, 1993