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Review

Comparative Histomorphological Review of Rat and Human Hepatocellular Proliferative Lesions

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Abstract: In this comparative review, histomorphological features of common nonneoplastic and neoplastic hepatocyte lesions of rats and humans are examined using H&E-stained slides. The morphological similarities and differences of both neoplastic (hepatocellular carcinoma and hepatocellular adenoma) and presumptive preneoplastic lesions (large and small cell change in humans and foci of cellular alteration in rats) are presented and discussed. There are major similarities in the diagnostic features, growth patterns and behavior of both rat and human hepatocellular proliferative lesions and in the process of hepatocarcinogenesis. Further study of presumptive preneoplastic lesions in humans and rats should help to further define their role in progression to hepatocellular neoplasia in both species. (DOI: 10.1293/tox.25.189; J Toxicol Pathol 2012; 25: 189–199)

Key words: liver, hepatocellular carcinoma, hepatocellular adenoma, large cell change, small cell change, foci of cellular alteration

Introduction

Primary hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most frequent cancer-related cause of death with increasing incidence worldwide[1–4]. In addition, HCC is the most common primary liver malignancy in the world[5–7]. In the majority of cases, it is associated with hepatitis B or C viral infections, aflatoxicosis, and/or liver cirrhosis[8–11]. Other risk factors for developing HCC include alcoholic liver disease, nonalcoholic steatohepatitis, diabetes, and obesity[12,13]. Most patients with HCC are diagnosed at a late stage; therefore, the prognosis of HCC patients is generally very poor, with a 5-year survival rate of less than 5%[7,14].

Experimental rat and mouse hepatocarcinogenesis models have been used for decades to delineate the pathogenesis of hepatic neoplasia. The rodent experimental model is used to identify potential human carcinogenic risk from exposure to drugs, environmental agents, and other xenobiotics. Rat hepatocellular adenomas (HCAs) and carcinomas are commonly used in tumor response and carcinogenicity bioassays and share some common features with human adenomas and carcinomas[15].

In rat experimental models, presumptive preneoplastic foci of cellular alteration occur prior to the appearance of hepatocellular adenomas and HCC; however, there is experimental evidence that not all foci of cellular alteration progress to neoplasia and that some may actually regress[16,17]. Basophilic (BAS), eosinophilic (EOS), and clear cell (CLEAR) foci of cellular alteration in rats are the counterparts of human liver cell dysplasias classified as large cell change and small cell change. The detection of these presumptive preneoplastic lesions in humans may be indicative of progression towards HCC[18–21] although further investigation is warranted. The purpose of this overview is to compare and contrast the morphological features of representative examples of commonly occurring human and rat hepatoproliferative lesions and to report the biology of these lesions.

Method

Paraffin blocks of adult human cases were selected from the archives of the Departments of Pathology, University Medical Center Utrecht (UMCU) and Erasmus Medical Center Rotterdam, The Netherlands. These surgical specimens were reviewed and considered unequivocal examples

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Table 1. H&E-stained Human (UMCU) / Rat (NTP) Liver Lesions

	Human			Rat					
		Gender %			Gender%				
Lesion	n	M	F	n	M	F			
FNH	12	8	92	–	–	–			
HCC	16	88	12	15	20	80			
HCA	15	–	100	10	40	60			
Dysplastic lesions	n	M	F	Foci of cellular alteration			n	M	F
LCC	19[1]	100	–	FCA/EOS			11	91	9
SCC	17[2]	78	22	FCA/BAS			9	89	11
				FGA/CLEAR			9	100	–

FNH, Focal Nodular Hyperplasia; HCA, Hepatocellular Adenoma; LCC, Large Cell Change; SCC, Small Cell Change; FCA, Focus of Cellular Alteration; EOS, Eosinophilic; BAS, Basophilic; CLEAR, Clear cell. [1] From 10 different patients; gender % is based on 10 people with at least one lesion. [2] From 9 different patients; gender % is based on 9 people with at least one lesion.

of human focal nodular hyperplasias (FNHs), HCCs, HCAs, large cell change (LCC) and small cell change (SCC).

Paraffin blocks of rat cases obtained from the National Toxicology Program (NTP) archives were from studies of chemical-induced liver tumors and represent diagnoses peer reviewed by experienced rodent toxicologic pathologists. Rat cases include HCCs, HCAs and basophilic, eosinophilic and clear cell foci of cellular alteration (FCAs). FNH lesions were not identified as they are rare in rats. However, this lesion was included in humans since it is one of the most common human proliferative liver lesions.

Original slides from the human and rat cases were reviewed by a medical liver pathologist and two toxicologic pathologists and selected using published diagnostic criteria[8,22–26] to confirm original diagnoses. Once confirmed, additional sections for this study were prepared, and all hematoxylin and eosin (H&E) staining was performed simultaneously at the UMCU after collection of all unstained paraffin slides on coated glass slides (e.g., Superfrost Plus) (see Table 1).

Results

Human cases

1. Focal nodular hyperplasia (FNH)

Liver samples were derived from surgical excision (hemihepatectomy, partial liver resection or biopsy) of female patients (11/12; 92%) and one male patient (1/12; 8%) at the UMCU. The corresponding resection specimens included in the study for comparison, were grossly nodular and ranged in diameter from 2 to 17 cm. Microscopically, they had classical diagnostic features of FNH consisting of nodules composed of plates of hyperplasic hepatocytes that were two-cell layers thick and subdivided by fibrous septa (Fig. 1). Thick-walled arteries were present in the stellate scars and septa, and there were bile ductules typically located between the scars and the liver parenchyma (Fig. 2). Near the fibrous septa there were occasionally small immature cells with oval to fusiform leptochromatic nuclei and scant cytoplasm that resembled rat oval cells. In addition, transitional cells displaying characteristics of both hepatocytes and bile duct cells were also present in some samples. These results suggest the presence of "undifferentiated progenitor cells" within FNH and further suggests that the ductular reaction, at least partly, can be explained by activation of these cells[27].

2. Hepatocellular carcinoma (HCC)

For the human samples, 14/16 HCC (88%) were from male patients. The morphological features consisted of a broad trabecular growth pattern of hepatocytes with occasional mixed growth patterns of trabecular/compact (Fig. 3), trabecular/acinar (Fig. 5) and sometimes a mixture of the three growth patterns. Hemorrhage, ischemic necrosis, neovascularization, angiectasis or peliosis hepatis and cystic changes were more commonly observed in these malignant tumors as compared to the other lesions evaluated.

3. Hepatocellular adenoma (HCA)

HCAs from female patients (n=15) had a maximum diameter of 16 cm. Histologically, they matched the common diagnostic criteria (see Table 2) for this benign liver neoplasm and sometimes showed focal to more diffuse steatosis, which can be observed in these tumors (Fig. 7)[28–30].

Human cases (continued)

1. Liver cell dysplasia – large cell change (LCC) and small cell change (SCC) (human)

Large cell change: LCC (synonyms: large liver cell change (LLCC) or large liver cell dysplasia (LLCD)) has been described in detail by Anthony *et al.*[31] and others[24,32–36]. Morphological features of hepatocytes with large cell change included cellular enlargement, nuclear pleomorphism with hyperchromasia, prominent nucleoli and occasional multinucleation. Enlargement was usually two- to three-fold and both nuclear and cytoplasmic with a normal nucleus to cytoplasmic ratio. Cytoplasmic staining was normal with occasionally more or less glycogen than that present in the surrounding liver parenchyma[31]. A classical example of such lesions is illustrated in Fig. 9. The selected cases of LCC (n=19) were all from 10 male patients.

Small cell change: SCC (synonyms: small liver

Table 2. Major Diagnostic Criteria for Proliferative Liver Lesions

Lesion	n	Definition
FNH	12	- Nodules of hyperplastic hepatocytes with two–cell layer hepatic plates divided by fibrous septa - Ductular reaction - Stellate scars with thick-walled arteries - Arising in a normal liver
HCC	16	- Trabecular or mixed growth patterns of atypical hepatocytes - Alteration of tinctorial staining patterns and marked cellular pleomorphism may occur - No portal tracts (unless entrapped by the normal liver) - Vascular/stromal invasion - Hemorrhage, necrosis, neovascularization, angiectasis and cystic changes more common than in HCA - Loss of normal reticulin framework - Isolated arteries - Mitotic index increased
HCA	15	- Proliferation of benign hepatocytes without acinar structures - Loss of normal lobular architecture - Compression of the surrounding parenchyma - Focal or diffuse steatosis - No more than 3 nodules/liver - Isolated arteries and arterioles - Mitotic index may be increased
Dysplastic lesions (human)		
LCC	19	- (Foci of) enlargement of hepatocytes 2- to 3-fold (both cytoplasmic and nuclear) - Nuclear pleomorphism with hyperchromasia - Prominent nucleoli - Multinucleation - Normal cytoplasm with less or more glycogen
SCC	17	- (Foci of) small hepatocytes with a high N:C-ratio - Nuclear atypia and different cytoplasmic staining - Fat or glycogen may differ from surrounding liver cells
Foci of cellular alteration (rat)		
FCA/EOS	11	- Normal or minimal compression of the surrounding parenchyma - (Foci of) enlarged, polygonal hepatocytes with (increased) acidophilic staining - Granular and pale intense eosinophilic cytoplasm of hepatocytes sometimes with a ground-glass appearance - Glycogen and/or some clear cells may be present
FCA/BAS	9	- Normal or minimal compression of the surrounding parenchyma - (Foci with) basophilic staining of hepatocytes of normal/smaller size - Cells sometimes arranged in tortuous cords and dissociation of cells may occur - Liver plates merge imperceptively with the surrounding parenchyma - Cells may be pleomorphic with enlarged (vesiculated) nuclei and prominent nucleoli
FCA/CLEAR	9	- Normal or minimal compression of the surrounding parenchyma - Normal/enlarged hepatocytes with acidophilic staining - Small nuclei, dense, centrally located - Excess storage of glycogen - Prominent cell membranes

FNH, focal nodular hyperplasia, HCA, hepatocellular adenoma, LCC, large cell change, SCC: small cell change, FCA: focus of cellular alteration; EOS, eosinophilic; BAS, basophilic; CLEAR, clear cell.

cell change (SLCC) or small liver cell dysplasia (SLCD)) was characterized by small hepatocytes with a high nuclear:cytoplasmic (N:C) ratio. Cells were uniform and differed from cells of the surrounding parenchyma in terms of nuclear atypia and cytoplasmic staining. Fat or glycogen content sometimes differed from that in the adjacent liver parenchyma. These collections of cells with small cell change tended to produce more small round distinct foci with irregular margins similar to foci that are more closely associated with the HCCs (Fig. 11) as reported by others[37–39]. The selected SCC cases (n=17) were from 9 patients (7/9, 78% males and 2/9, 22% females). In the cases evaluated, combined areas of LCC and SCC could sometimes be observed within the same slide.

Rat cases

1. Hepatocellular carcinoma (HCC)

The HCCs reviewed, were from dosed males (3/15 HCC, 20%) and females (12/15, 80%). The morphological features were consistent with published HCC criteria[26]

and exhibited largely trabecular growth patterns, although mixed growth patterns (trabecular/acinar/solid) and occasionally basophilic and eosinophilic areas were present. In one case, the HCC arose within a hepatocellular adenoma and had an infiltrative growth pattern. A trabecular growth pattern with focal steatosis and mitosis is illustrated in Figure 4. As was seen in human HCCs, sometimes areas with acinar growth patterns were observed (Fig. 6). Most lesions evaluated also had hemorrhage, necrosis, pigment deposition (hemosiderin), angiectasis and/or focal fatty change.

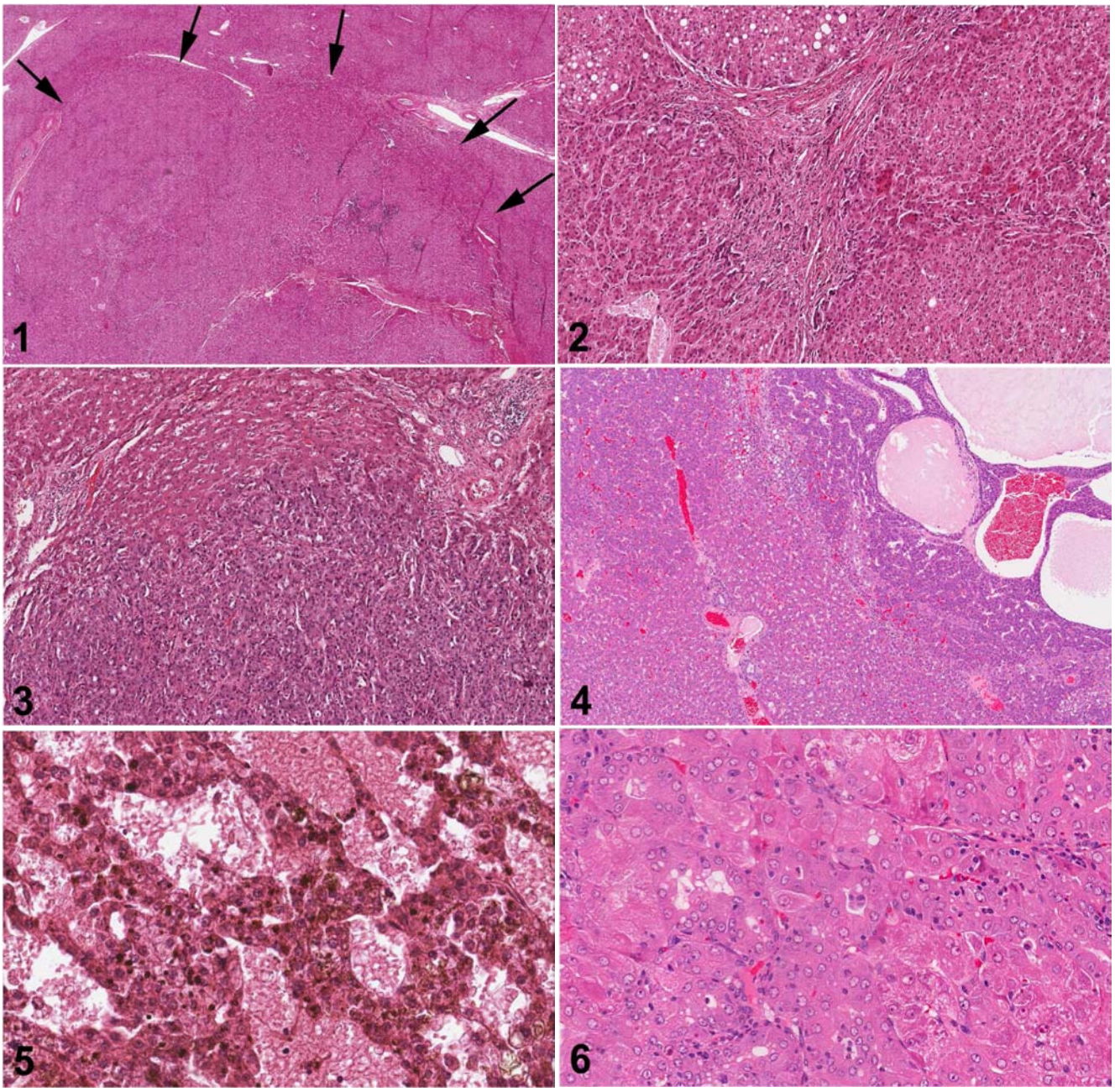


Fig. 1. Human liver. Low magnification of FNH. The upper border of the FNH is indicated by arrows with relatively normal hepatic parenchyma at the top of the figure. H&E.

Fig. 2. Human liver. Higher magnification of Fig. 1. The FNH consists of nodules composed of two-cell layers of hepatocytes subdivided by fibrous septa. Proliferative ductules are present in stellate septal scars. H&E.

Fig. 3. Human liver. Hepatocellular carcinoma composed of atypical hepatocytes arranged in a solid or trabecular growth pattern with normal hepatic parenchyma present at the top of the figure. H&E.

Fig. 4. Rat liver. Hepatocellular carcinoma with a trabecular growth pattern at the top and right of the figure. There is angiectasis in the carcinoma on the right. Normal hepatic parenchyma is present on the lower left of the figure. H&E.

Fig. 5. Human liver. High magnification of a hepatocellular carcinoma with a mixed acinar and trabecular growth pattern. H&E.

Fig. 6. Rat liver. High magnification of a hepatocellular carcinoma with a mixed acinar and trabecular growth pattern. H&E.

2. Hepatocellular adenoma (HCA)

The selected hepatocellular adenomas (n=10) were from animals of both sexes (6/10, 60% females and 4/10, 40% in males). The histological features were compatible with the common diagnostic criteria (see Table 2). Sometimes, these adenomas also contained diffuse fatty change (steatosis) and angiectasis (Fig. 8).

3. Focus of cellular alteration (FCA)

Eosinophilic foci: Eosinophilic foci of cellular alteration consisted of polygonal enlarged hepatocytes with increased acidophilic staining compared with the surrounding normal liver. Clear cells (glycogen storage) were occasionally present. The cytoplasm was distinctly smooth to sometimes granular and pale pink, with a "ground-glass" appearance. Nuclei were enlarged, and nucleoli were prominent and centrally located. A classical example is illustrated in Fig. 10. For the eosinophilic foci evaluated (n=11), 10/11 (91%) were in male rats.

Basophilic foci: Basophilic foci may show some resemblance with small cell change as observed in humans. Different subtypes have been described[26]. The tigroid subtype consists of a focus of (small) basophilic cells distinct from the surrounding liver parenchyma and arranged in tortuous cords. Cells display large abundant basophilic bodies often arranged in clumps or long bands with a striped ("tigroid") pattern in the paranuclear or peripheral regions of the cytoplasm (due to increased rough endoplasmic reticulum). In the samples we evaluated, the diffuse subtype was most common and consisted of small, discrete, clearly demarcated, strongly basophilic foci with irregular borders. These foci were randomly distributed within the hepatic lobule. Although different subtypes have been recognized in rodents, a typical example is shown in Fig. 12. Of the basophilic foci evaluated (n=9), 8/9 (89%) were observed in male rats.

Clear cell foci: Clear cell areas of groups of hepatocytes can be observed in HCCs in both human and rodents[40–43]. Clear cell foci consisted of normal or enlarged groups of cells with prominent cell membranes and distinct cytoplasmic clear spaces surrounding a densely stained centrally located nucleus. Some eosinophilic or basophilic cells were occasionally present within clear cell foci. A classical example of a clear cell focus is presented in Fig. 13.

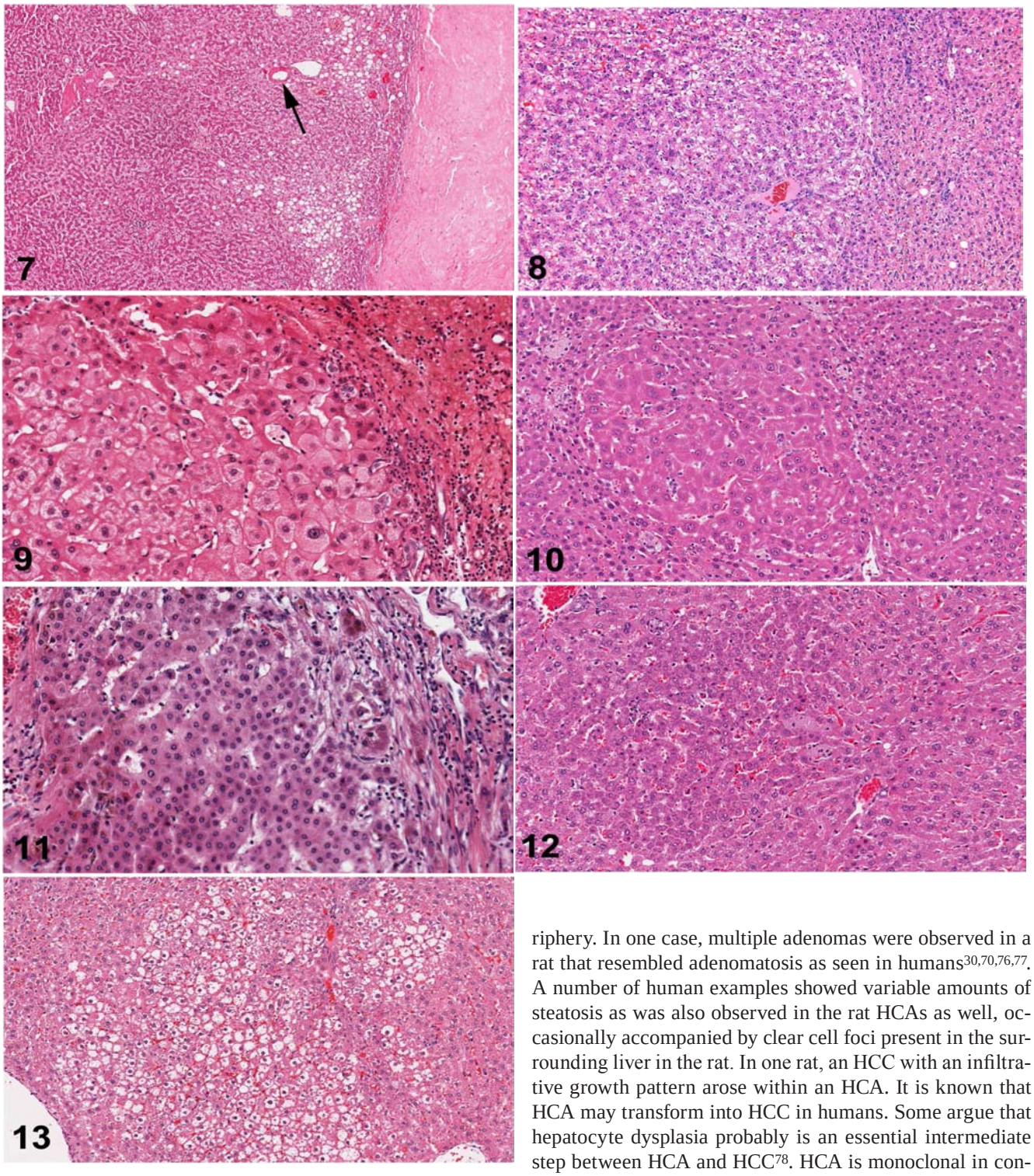
The role of clear cell foci in hepatocarcinogenesis is elusive and poorly described, although metabolic changes in carbohydrate metabolism have been associated with HCCs in both humans and rodents[41,42,44,45], and therefore these foci, as observed in the rat liver, could play a role in liver tumor formation. The selected clear cell foci (n=9) were only found in the livers of male rats.

Discussion and Conclusion

The liver is a major target organ in preclinical toxicity and oncogenicity safety assessment studies with rodents. The significance of hepatic neoplastic findings in animal models has been questioned with regard to their predictive value, as humans appear resistant to many agents that readily produce liver tumors in rodents[46]. As toxicity to the liver is reported to be the second most frequent cause of drug failure due to adverse effects in clinical trials of potential drugs[15,47,48], the early detection and interpretation of proliferative lesions as well as nonproliferative hepatic lesions is of vital importance. One of the safety issues after long-term administration of a xenobiotic is carcinogenicity assessment, and both early and late proliferative liver lesions might be indicative of potential hepatocarcinogenesis or carcinogenesis at other sites in humans[1,16,37,49–54]. The human and rat cases selected for this review were all morphologically consistent with descriptive features in texts and the peer reviewed literature using the most common and contemporary diagnostic criteria for both human and rat hepatic lesions[8,22–24,26].

We have reviewed FCAs (eosinophilic, basophilic and clear cell foci) and compared them with the human counterparts of LCC and SCC. LCC and SCC in humans and eosinophilic and basophilic FCA in the rat showed common histomorphological characteristics (Figs. 9 to 12), which might be indicative of a mutual presumptive role in the process of hepatocarcinogenesis. FNH is the second most common benign lesion of the liver in humans and occurs more often in young females but can occur in both sexes of adults[55–60]. Cases in children have been reported[61–63]. FNH is a benign lesion, and in contrast to HCA, the risk of complications such as hemorrhage and malignant transformation is virtually absent. The more common occurrence of FNH among young women is in line with our results that show that nearly all FNHs selected for this study were obtained from women (91.7%). FNH is a rare lesion in animals[64,65]. Immunohistochemistry showed characteristic mild and focal cytokeratin 7 staining of hepatocytes, whereas cytokeratin 7 and cytokeratin 19 show a strong staining of bile ductules in the fibrous septa[39]. The majority of patients with FNH have normal liver test results and alpha-fetoprotein is mostly in the normal range[66]. Diagnosis of FNH may be difficult in humans if one or more major features are not prominent (central scar, ductular reaction) or if the FNH is steatotic, or has small nodules[57]. A clear overview of the clinical and morphological features of FNH is presented by Ferrell and Kakar[24] for distinction between FNH, hepatocellular adenoma and HCC. Immunohistochemistry has also been extensively investigated and can be supportive for the differential diagnosis[10,28,39,67]. The current opinion regarding the etiology of this lesion is that it represents a hyperplastic and altered growth of hepatocytes surrounding a pre-existing arterial malformation in response to changes in blood flow in the parenchyma[8,23,68]. Treatment of symptomatic FNH in humans consists first of embolization and then resection[69].

Following hepatic angioma and FNH, HCA is the third most common benign proliferative lesion in humans and is known to occur in 85% of young female patients taking oral contraceptives[70,71]. There is considerable overlap in morphologic features of well-differentiated hepatocellular lesions, necessitating the use of immunohistochemistry and other



techniques for diagnosis[39,72–75]. More current diagnostic criteria distinguish four different subtypes of human HCAs based on their histological and molecular characteristics[29]. The human HCAs examined were all from female patients, in line with the epidemiology and common occurrence of these tumors. HCA histological features observed in the rat were similar to those in humans. The tumors showed focal fatty change/steatosis and increased numbers of mitoses and there was also normal pre-existing liver present at the periphery. In one case, multiple adenomas were observed in a rat that resembled adenomatosis as seen in humans[30,70,76,77]. A number of human examples showed variable amounts of steatosis as was also observed in the rat HCAs as well, occasionally accompanied by clear cell foci present in the surrounding liver in the rat. In one rat, an HCC with an infiltrative growth pattern arose within an HCA. It is known that HCA may transform into HCC in humans. Some argue that hepatocyte dysplasia probably is an essential intermediate step between HCA and HCC[78]. HCA is monoclonal in contrast to FNH (polyclonal) and consequently has an inherent risk for progression to HCC[78]. Moreover, ß-catenin-mutated HCA has an increased risk of undergoing malignant change[79]. Both the human and rat hepatocellular carcinomas reviewed in this study had malignant growths pattern that are typical for these tumors. Microscopically, the WHO distinguishes trabecular, acinar (pseudoglandular), scirrhous and solid forms[8]. Special histological subtypes, not included in this review, are the clear cell, fibrolamellar and mixed hepatocholangiocellular variants. Trabecular growth pat-

terns were the most common in both human and rat HCCs (Figs. 3 and 4) evaluated; sometimes mixtures of trabecular, solid and acinar/pseudoglandular patterns (Figs. 5 and 6) were also present in our collection. Both human and rat HCCs showed common histopathological characteristics that are typical for these malignant liver lesions. Liver cell dysplasia (LCD) is described in liver transplants containing underlying liver disease. These dysplastic hepatocytes can frequently be observed in the cirrhotic liver[19,80,81] and have been proposed to contain precancerous properties[31,38,82]. The cytological criteria for the diagnosis of LCD include cytoplasmic and nuclear changes, nuclear crowding or pleomorphism together with prominent nucleoli, hyperchromasia and sometimes multinucleation. The cytological features of liver cell dysplasia can strikingly mimic HCC[83] suggesting it is a putative preneoplastic lesion that can precede HCC in various species[1,10,41,45,84–87]. The precancerous nature of both LCC and SCC with regard to progression to HCC is somewhat controversial, but some claim that either one or both of them have been associated with development of HCC[31,35,38,49,82,87–90].

In rats, FCAs have likewise been designated to play a precursor role in the process of hepatocarcinogenesis as they represent a localized proliferation of hepatocytes that are phenotypically different from the surrounding liver. These FCAs occur spontaneously in aged rats and other rodents and can be induced by chemical treatment. The incidence of spontaneous foci is highest in rats and can reach nearly 100% in F344 rats by the age of 2 years[91]. After administration of hepatocarcinogens, their incidence, size and/or multiplicity are usually increased, and latency usually decreased[25,41,86]. These foci have been described in a number of animal models and are considered as precursor lesions of HCC[45], but controversy still remains. Some of these foci may have autonomous growth potential and may show enzyme profiles different from the normal hepatocytes (e.g., positive for γ-glutamyl transpeptidase, α-fetoprotein, glutathione S-transferase placental form, and negative for glucose-6-phosphatase and glycogen phosphorylase). However, it has been shown that certain conditions are required for promotion and progression of initiated cells. In addition, different mechanisms of promotion by different chemicals have been demonstrated in the multistep process of carcinogenesis, and it is stated that not all foci become neoplasms[91–94]. Some foci of cellular alteration can even regress, and different types of foci have different potentials for developing into neoplasms[17,95].

Since controversy with regard to the significance of presumptive preneoplastic liver lesions still exists, comparative research of these proliferative lesions in both rats and humans is needed.

Based on comparison of the histomorphological features of common nonneoplastic and neoplastic hepatocyte lesions of rats and humans, it is apparent that there are major similarities in diagnostic features, growth patterns, and behavior of these lesions in both species. Further study of presumptive preneoplastic lesions should help to further define their role in progression to malignancy and to provide a basis for using liver responses in rodents exposed to xenobiotics in safety assessment studies to predict potential risks to humans. Morphological similarities as illustrated in this review will be a first step to understanding their significance and relevance in human and animal liver tumor formation.

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Fig. 7. Human liver. Hepatocellular adenoma with multifocal steatosis and thick-wall blood vessel (arrow). Prominent fibrosis is present on right edge of the figure. H&E.

Fig. 8. Rat liver. Hepatocellular adenoma. The adenoma is sharply demarcated with slight compression of the adjacent normal parenchyma. H&E.

Fig. 9. Human liver. A classic example of large cell change. H&E.

Fig. 10. Rat liver. Eosinophilic focus of cellular alteration consisting of enlarged hepatocytes with increased acidophilic staining compared to the surrounding hepatic parenchyma. H&E.

Fig. 11. Human liver. Small cell change comprised of a focus of small cells with an irregular margin within a cirrhotic liver. H&E.

Fig. 12. Rat liver. A basophilic focus of cellular alteration with irregular but discrete margins surrounded by more eosinophilic normal parenchymal hepatocytes. H&E.

Fig. 13. Rat liver. Clear cell focus of cellular alteration with an irregular border and comprised of hepatocytes with clear cytoplasm and a centrally located nucleus. H&E.

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