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Classification of primary hepatic tumours in the cat

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ABSTRACT

Hepatic tumours in dogs have recently been re-classified to follow a revised human classification system that takes account of identified hepatic progenitor cells. This study investigated the presence and relative frequency of morphological types of feline primary hepatic neoplasms and aimed to determine whether a similar new classification scheme could be applied in cats. Feline primary liver tumours ($n = 61$) were examined histologically and with a series of immunohistochemical markers.

Six cases of nodular hyperplasia and 21 tumours of hepatocellular origin were diagnosed. The latter were subdivided into hepatocellular tumours that were well differentiated and had no evidence of metastases ($n = 18$) and tumours that showed poorly differentiated areas with marked cellular and nuclear pleomorphism and had intrahepatic and, or, distant metastases ($n = 3$). These malignant feline hepatocellular tumours maintained their hepatocellular characteristics (HepPar-1, MRP2, pCEA positive) and were negative, or only <5% positive, for K19. Twenty-five cholangiocellular tumours were diagnosed and all had intrahepatic and, or, distant metastases. Eight NSE positive small cell carcinomas (carcinoids) were diagnosed and subdivided into small cell carcinomas with HPC characteristics (K19 positive) and neuroendocrine carcinomas (K19 negative). In addition, one squamous cell carcinoma originating from the distal part of the choledochal duct was recognised.

Feline primary hepatic neoplasms can be sub-divided into benign and malignant hepatocellular tumours, cholangiocellular carcinomas, small cell carcinomas with HPC characteristics, neuroendocrine carcinomas and squamous cell carcinomas. The marked species difference justifies a specific classification for feline primary hepatic neoplasms.

Introduction

Primary liver tumours represent 1.0–2.9% of all tumours in cats (Hammer and Sikkema, 1995; Hall et al., 2005). Currently, feline hepatic neoplasms are classified as hepatocellular adenomas and carcinomas, cholangiocellular adenomas and carcinomas, mixed hepatocellular and cholangiocellular carcinomas and hepatic carcinoids (Patnaik, 1992; Cullen and Popp, 2002; Charles et al., 2006); a single feline hepatoblastoma has been described in the literature (Ano et al., 2011).

Many advances have been made in the characterisation of primary liver tumours in humans, in particular relating to the identification and role of hepatic progenitor cells (HPCs). HPCs are multipotent cells, located within the canals of Hering, with the capacity for self-renewal and differentiation into mature hepatocytes and cholangiocytes. In cases that have severe liver cell damage, for example in acute fulminant hepatitis, HPCs proliferate, and can be identified as keratin (K) 19 positive ductular proliferation in the periportal areas (Libbrecht and Roskams, 2002; Turner et al., 2011; Boulter et al., 2012). HPCs and their prognostic role were also identified in primary hepatocellular tumours in humans: tumours with HPC characteristics were poorly differentiated and aggressive (Allison and Lovell, 2005; Libbrecht, 2006; Roskams, 2006). Studies on the role of HPCs in the development and differentiation of various hepatic tumours have resulted in a new histomorphological and immunohistochemical classification of primary liver tumours in humans, taking into account their aggressiveness and prognosis (Komuta et al., 2008, 2012) (Fig. 1).

Recently, a comparable classification of primary liver tumours in dogs has been made, whereby hepatocellular carcinomas and cholangiolocarcinomas with HPC characteristics were recognised (van Sprundel et al., 2010, 2013). Primary hepatic neoplasms in the cat can also be classified and differentiated using morphological and immunohistochemical characteristics (Patnaik, 1992).

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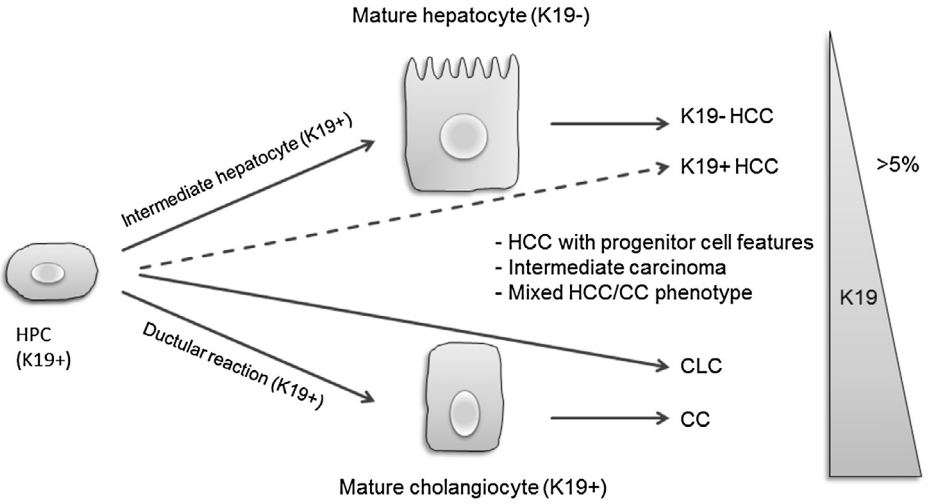

Fig. 1. Proposed origin and classification of primary liver tumours in humans. Primary liver tumours may arise from mature hepatocytes or cholangiocytes representing the classic hepatocellular and cholangiocellular adenomas and carcinomas. They may also arise from hepatic progenitor cells showing varying degrees of differentiation and develop various and sometimes overlapping morphological features such as cholangiolocarcinomas. HPC, hepatic progenitor cell; HCC, hepatocellular carcinoma; CC, cholangiocellular carcinoma; CLC, cholangiolocarcinoma; K19, keratin 19. From van Sprundel et al. (2013).

The aim of the present study was to investigate the occurrence and relative frequency of morphological types of primary hepatic neoplasms in the cat and to determine whether a classification similar to the canine scheme can be applied to cats.

Material and methods

Samples

Formalin-fixed paraffin-embedded material from primary liver tumours of 61 cats was available from the archives of the Department of Pathobiology, Utrecht University ($n = 7$), Valuepath Laboratory for Veterinary Pathology, Hoensbroek, The Netherlands ($n = 7$), the Institute of Veterinary Pathology, University of Zürich, Switzerland ($n = 35$), and the Institute of Veterinary Pathology, Free University Berlin, Germany ($n = 12$). All material was derived from clinical cases and had been submitted for individual diagnostic purposes; no tissue was collected specifically for the purpose of the present study. Formalin-fixed paraffin-embedded samples from the livers of healthy cats and a cat with reactive ductular proliferation associated with acute fulminant hepatitis, as well as a sample from an adrenal gland of a cat, were also available from the Department of Clinical Sciences of Companion Animals, Utrecht University.

Grading and staging

Histological grading and staging of the tumours was performed as described by van Sprundel et al., (2010, 2013). The parameters scored from 0 to 3 for grading comprised cell and nuclear pleomorphism, presence or absence of multinucleated tumour cells and mitotic activity. Three stages were based on histopathology, anamnestic data (ultrasonography and surgery reports), including follow-up data from referring veterinarians and/or owners and post-mortem pathology reports. These were: (1) Stage 0, macroscopically only one tumour process was present in the liver and, or, microscopically the tumour was well circumscribed or encapsulated; there was no evidence of intrahepatic or extrahepatic metastases; (2) Stage 1, microscopically the tumour had spread beyond the original (primary) site to the adjacent tissue, there was evidence of intravascular spread or the presence of microsatellites and/or multiple tumour processes were present in the liver macroscopically; (3) Stage 2, the tumour had spread from the primary site to the regional lymph nodes and/or other organs (distant metastases).

Immunohistochemistry

Immunohistochemistry was performed for keratin 19 (K19), HepPar-1, multidrug resistance-associated protein 2 (MRP2), polyclonal carcinoembryonic antigen (pCEA), neuron-specific enolase (NSE), and chromogranin-A (Cg-A) (Table 1). The immunohistochemical reactions (IHCs) were optimized for the cat and accepted for this investigation when the immunohistochemical staining pattern complied with the information described in the literature and the information provided by the manufacturer of the antibodies.

Normal healthy feline liver served as positive control for K19, HepPar-1, MRP2 and pCEA; the liver with fulminant hepatitis was used to demonstrate positive staining of the ductular (HPC) proliferation for K19. Adrenal glands served as a positive control for NSE and Cg-A. Negative controls were performed by replacing the primary antibody with antibody diluent.

Results

In the normal liver, hepatocytes showed moderate to marked cytoplasmic staining for HepPar-1 (Fig. 2a), mild to marked canalicular staining for MRP2 (Fig. 2b) and mild to marked canalicular, and sometimes slight cytoplasmic, staining for pCEA (Fig. 2c). Bile ducts showed strong cytoplasmic staining for K19 (Fig. 2d). The small bile ducts were negative or showed slight cytoplasmic and, or, apical staining for pCEA whereas the larger bile ducts showed

Table 1
Antibody characteristics and experimental procedures for immunohistochemistry.

Antibody	Manufacturer	Type	Clone	Antigen retrieval	Dilution	Wash buffer	Incubation
K 19	Novocastra	Mouse mAb	b170	Proteinase K	1:100	TBS	O/N 4 °C
HepPar-1	Dako	Mouse mAb	OCH1E5	Tris/EDTA	1:50	PBS	O/N 4 °C
MRP2	Monosan	Mouse mAb	M_2III-6	Citrate	1:300	PBS	O/N 4 °C
pCEA	Dako	Rabbit pAb	Code nr; A0115	Tris/EDTA	1:500	PBS	O/N 4 °C
NSE	Dako	Mouse mAb	BBS/NC/VI-H14	Citrate	1:400	PBS	O/N 4 °C
Cg-A	Immuno Star	Rabbit mAb	20086 SP-1	No antigen retrieval	1:800	PBS	O/N 4 °C

Cg-A, chromogranin-A; EDTA, ethylene diamine tetraacetic acid; pCEA, polyclonal carcinoembryonic antigen; K19, Keratin 19; MRP2, multidrug resistance-associated protein 2; mAb, monoclonal antibody; NSE, neuron-specific enolase; O/N, over-night; PBS, phosphate-buffered saline; pAb, polyclonal antibody; Prot K, proteinase K; RT, room temperature; TBS, Tris-buffered saline.