J Toxicol Pathol 2011; 24: 125-129

Case Report

Focal Nodular Hyperplasia in the Livers of Cynomolgus Macaques (*Macaca Fascicularis*)

Junko Fujishima[1], Shigeru Satake[1], Tomohiro Furukawa[1], Chika Kurokawa[1], Rinya Kodama[1], Akiko Moriyama[1], Yuji Sasaki[1], Yasuhiro Kamimura[1], and Hiroshi Maeda[1]

[1] Shin Nippon Biomedical Laboratories, Ltd., 2438 Miyanoura, Kagoshima 891-1394, Japan

Abstract: Two cases of spontaneous focal hepatic hyperplasia were observed in young female cynomolgus macaques (*Macaca fascicularis*). Grossly, a single raised nodule was observed in the left hepatic lobe. Histopathologically, the nodule compressed surrounding normal tissue; however, the hepatic cords within the nodule continued to those in the normal area except in part. Extensive fibrosis and absence of a normal hepatic triad were observed in the nodule. Thin fibrous septa radiating from the dense central stellate scarring and distended vessels were apparent in one animal. Hepatocytes in the nodule lacked cellular atypia, showed frequent PAS-positive eosinophilic inclusions in the cytoplasm and showed higher positive ratios for PCNA. The present cases resembled focal nodular hyperplasia reported in humans and a chimpanzee. (DOI: 10.1293/tox.24.125; J Toxicol Pathol 2011; **24**: 125-129)

Key words: liver, cynomolgus macaque, focal nodular hyperplasia

Nonneoplastic inflammatory lesions such as cellular infiltration and parasitic lesions are frequently observed in the livers of nonhuman primates[1,2]. On the other hand, neoplastic and nonneoplastic proliferative lesions are rare, and only one case of nonneoplastic proliferative lesion has been reported in the chimpanzee[3] (Table 1). In this report, we describe the histopathology of unusual spontaneous hepatocellular hyperplasia in two young cynomolgus macaques.

The animals were naïve female cynomolgus macaques (*Macaca fascicularis*, purpose-bred) born in China. Case 1 was three years old, and Case 2 was four years old. The animal room was maintained at a temperature range of 23 °C to 29 °C and a humidity range of 30% to 70%, with air ventilation 15 times/hour and artificial illumination for 12 hrs/day (07:00 to 19:00). The animals were individually housed in stainless steel cages (680 mm deep × 620 mm wide × 770 mm high) and provided with approximately 108 g of chewable animal feed for nonhuman primates (HF Primate 5K91 12G 5K9J, Purina Mills, LLC., St. Louis, MO, USA). Water, certified to meet the water quality standards required by the Japanese Waterworks Law was available *ad libitum* from an automatic supply system (Edstrom Industries, Inc., Waterford, WI, USA). All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Shin Nippon Biomedical Laboratories, Ltd. and were performed in accordance with standards published by the National Research Council (Guide for the Care and Use of Laboratory Animals, NIH OACU) and the US National Institutes of Health Policy on Humane Care and Use of Laboratory Animals. In accordance with these standards, every effort was made to ensure that the animals were free of pain and discomfort. No abnormal clinical signs were observed before necropsy, and no abnormal findings were observed in the hematology, blood chemistry or urinalysis. The animals were anesthetized by an intravenous injection of sodium pentobarbital and euthanized by exsanguination. After macroscopic observation, the liver was fixed in 10% neutral buffered formalin and processed for routine paraffin embedding. The paraffin sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff stain (PAS), Masson's trichrome stain (MT) and silver impregnation for reticulin fiber. For immunohistochemistry, a mouse monoclonal antibody against proliferating cell nuclear antigen (PCNA, Dako Japan, Kyoto, Japan) was used. The paraffin sections were deparaffinized and processed for immunohistochemistry (Envision+ Mouse/HRP, Dako Japan, Kyoto, Japan). Small sections of 3% glutaraldehyde-fixed liver tissue were further fixed in 1% osmium tetroxide and processed for transmission electron microscopy.

Grossly, single, raised spherical nodules with diameters of 30 mm in Case 1 (Fig. 1) and 5 mm in Case 2 were observed in the left lobe. The nodules were paler than the surrounding tissue. The cut surface of the nodule in Case 1 was swollen. There were no abnormal gross lesions other than in the liver.

The histopathological features of the nodules in both animals essentially resembled each other. Common findings

Received: 28 October 2010, Accepted: 27 December 2010
Mailing address: Junko Fujishima, Shin Nippon Biomedical Laboratories, Ltd., 2438 Miyanoura, Kagoshima 891-1394, Japan
TEL: 81-99-294-2600 FAX: 81-99-294-3619
E-mail: fujishima-junko@snbl.co.jp

Table 1. Spontaneous Hepatic Neoplastic and Nonneoplastic Proliferative Lesions of Monkeys

Hepatic lesion	Species	Number of case	Age (year)	Reference
Neoplastic lesion				
Hepatocellular carcinoma	*Cercocebus atys*	1	≒10	4
	Lemur macaco	1	Unknown	5
	Macaca fascicularis	2	<5	6
	Macaca fascicularis	1	5	7
	Macaca mulatta	1	Unknown	8
	Pan troglodytes	1	≒12	9
	Pan troglodytes	2	>7	8
	Saimiri bolivinsis	1	>24	10
	Saimiri sciureus	1	>13	11
Hepatocholangiolar carcinoma	*Macaca fascicularis*	1	<5	6
	Cercopithecus aethiops	1	Adult	12
Cholangiocarcinoma	*Cebus albifrons*	1	25	13
	Lemur catta	1	>18	14
Hepatic myelolipoma	*Callithrix jacchus*	1	2.7	15
Hepatocellular adenoma	*Presbytis entellus*	1	Unknown	5
	Cercopithecus aethiops *Macaca mulatta* *Papio hamadryas* *Presbytis entellus*	11	Unknown	16
Nonneoplastic proliferative lesion				
focal nodular hyperplasia	*Pan troglodytes*	1	23	3

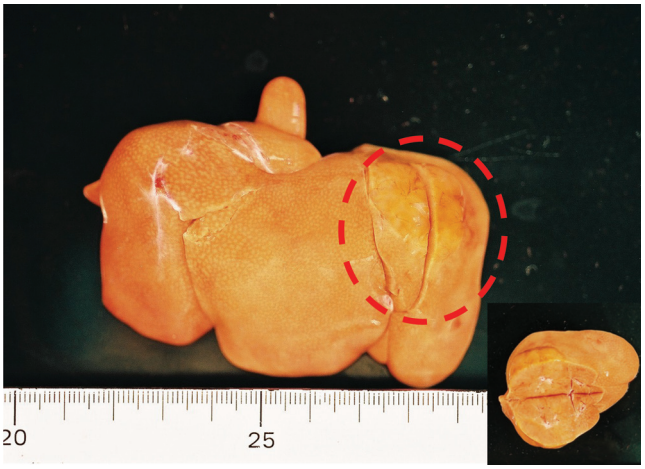

Fig. 1. Gross appearance of the liver. The nodule (enclosed by a broken line) was observed in the left lobe. Inset: the nodule was swollen on the cut surface. Case 1.

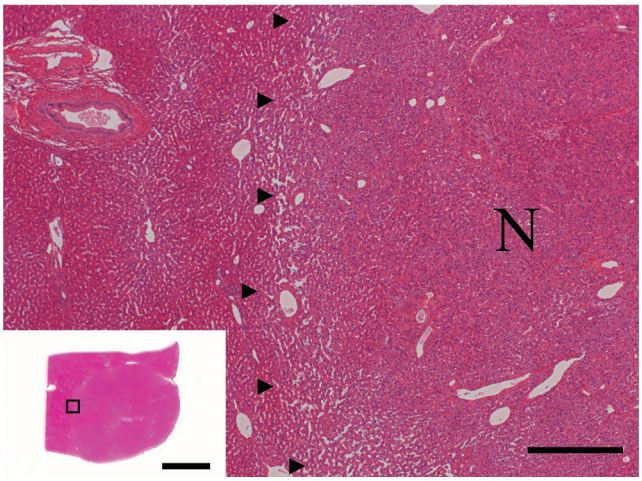

Fig. 2. The nodule (N) was discernable by compression of adjacent normal tissue (border marked with arrowheads). Bar = 500 µm. Inset: low power view of the nodule (N). Bar = 10 mm, Case 1, HE.

were as follows: The boundary of the nodules were indistinct, and the hepatic cords in the nodules continued to those in the normal hepatic area. The nodules were discernable by compression of adjacent normal tissue with slightly dilated sinusoid and partial encapsulation (Fig. 2). Extensive fibrosis was prominent in the nodules and divided the hepatic lobules. Bile ducts and blood vessels were observed in the fibrosis; however, the bile ducts were not apparent except

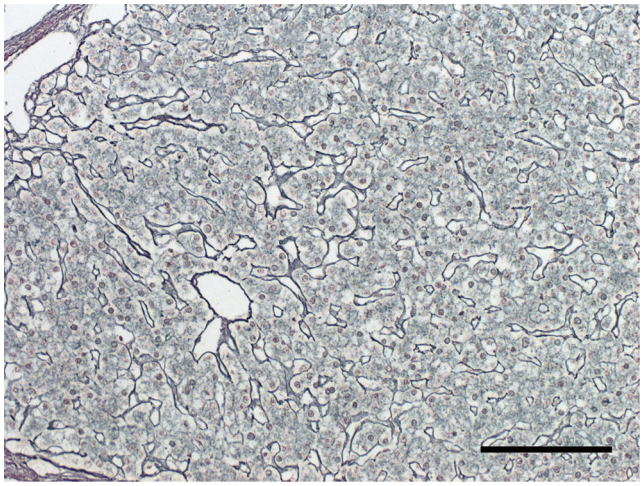

Fig. 3. Hepatic cords were partially disordered and composed of two or three cell-layer thick hepatocytes. Case 1, silver impregnation. Bar = 100 µm.

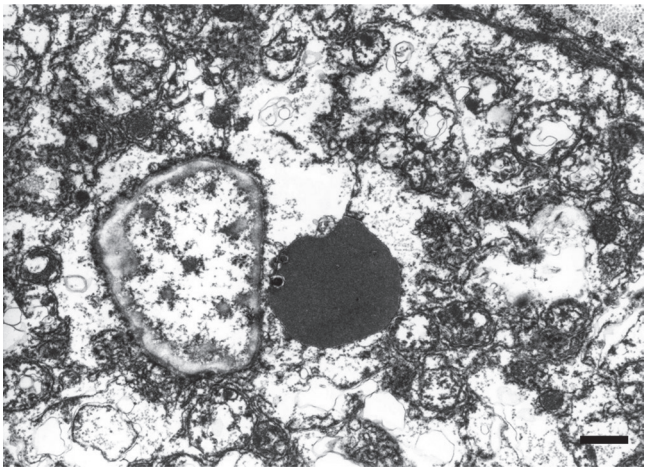

Fig. 5. The inclusion was membrane-bound homogeneous electron dense material. Case 1, transmission electron micrograph. Bar = 1 µm.

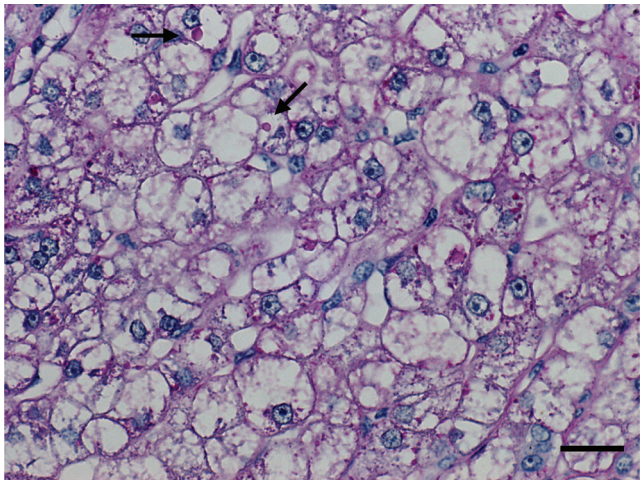

Fig. 4. PAS-positive intracytoplasmic inclusions (arrows) in the hepatocytes. Case 1, Periodic acid-Schiff. Bar = 20 µm.

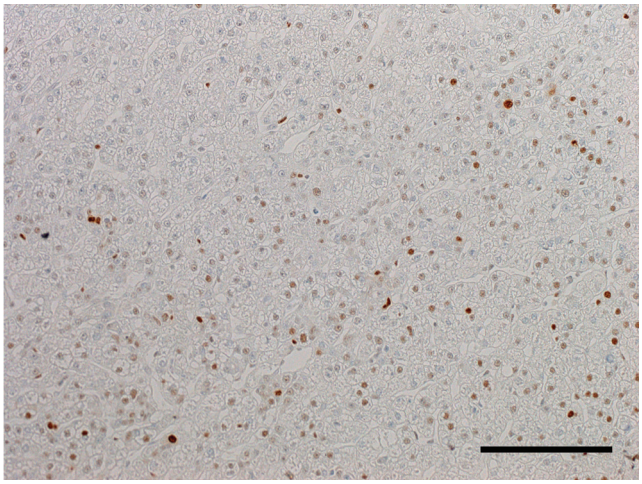

Fig. 6. Numerous PCNA-positive hepatocytes. Case 1, anti-PCNA immunohistochemistry. Bar = 100 µm.

in the fibrosis area. Absence of a normal hepatic triad was observed in the nodules. The hepatic cords were partially disordered and composed of two or three cell-layer thick hepatocytes (Fig. 3). Hepatocytes in the nodules lacked cellular atypia, such as pleomorphism, nucleus/cytoplasm ratio and polarity. PAS-positive eosinophilic inclusions, similar in size to erythrocytes, were frequently observed in the hepatocytic cytoplasm (Fig. 4). Electron microscopy showed the inclusion to be membrane-bound homogeneous electron dense material (Fig. 5). Although increased mitosis was not apparent in the HE sections, hepatocytes in the nodules showed a higher positive ratio for PCNA than in normal areas (Fig. 6).

In Case 1, thin fibrous septa radiated from the central dense stellate scar. (Fig. 7). The fibrous septa irregularly separated the hepatic lobules and partially formed a pseudolobule-like structure (Fig. 8). Bile ducts and distended blood vessels were abundant in the central dense stellate scar. In some areas, hepatocytes showed large and clear cytoplasms (Fig. 4). Nucleoli were prominent, and binucleated hepatocytes were frequently observed in the area.

In Case 2, fibrosis was much less, and the stellate scar was obscure (Fig. 9). Fibrous tissue partially entered into the hepatic lobules and irregularly separated the hepatic cords, where the basic lobular structures, such as the central vein and portal triad, were lost. Hepatocytes labeled with anti-PCNA antibody were more numerous than in Case 1.

It is unlikely that the lesions in the present cases were responses to inflammatory change, such as hepatitis or cholangitis, because they were focal, lacked apparent inflammatory cell infiltration and showed expansive proliferation.

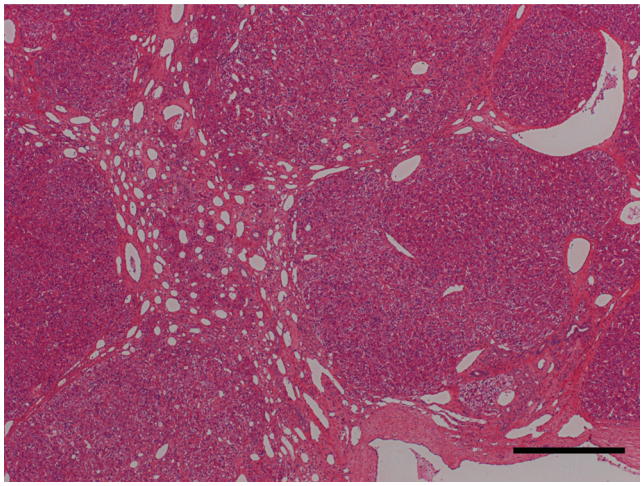

Fig. 7. Fibrous septa radiated from a central stellate scar, known as a "wagon-wheel" appearance. Case 1, HE Bar = 500 µm.

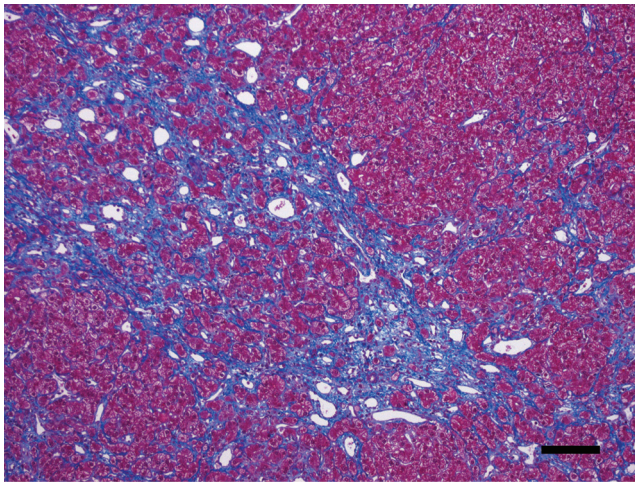

Fig. 9. Fibrous tissue partially entered into the hepatic lobules and irregularly separated the hepatic cords. Case 2, Masson's trichrome. Bar = 100 µm.

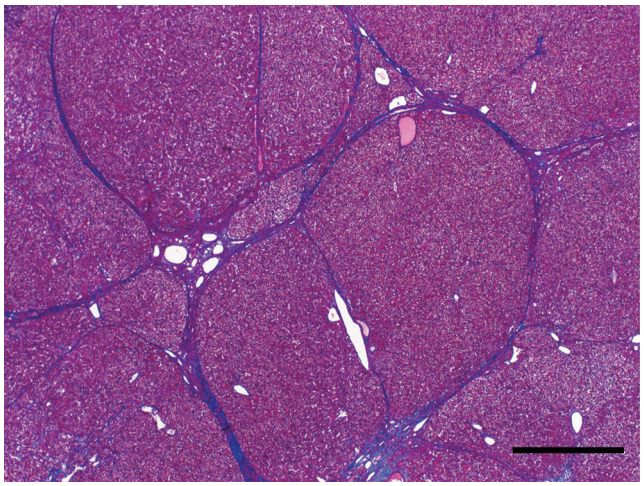

Fig. 8. The fibrous septa irregularly separated the hepatic lobules forming a pseudolobule-like structure. Case 1, Masson's trichrome. Bar = 500 µm.

The high PCNA-positive rate of the hepatocytes showed high proliferative activity in the nodules. However, the lesions were considered not to be neoplasia but to be hyperplasia because apparent cellular atypia was lacking in the hepatocytes, the basic lobular structure was maintained and the hepatic cords in the nodules continued to those in normal hepatic areas. Therefore, the nodules were considered to be nonneoplastic hyperplastic lesions.

Tumor-like lesions in the livers of domestic animals are classified as *nodular hepatocellular hyperplasia* (similar lesions are also known as *nodular hyperplasia* or *hyperplastic nodule*) and *regenerative hepatocellular hyperplasia*[17–21] and are classified in humans as *focal nodular hyperplasia* and *nodular regenerative hyperplasia*[22,23].

Nodular hepatocellular hyperplasia occurs frequently in aged dogs[17–19] and has been also reported in swine at a low incidence[17,20]. There are many morphological differences between the present cases and canine nodular hepatocellular hyperplasia. Canine nodular hepatocellular hyperplasia is characterized by randomly distributed multiple distinct nodules and occurs throughout. Although compression of surrounding normal tissue by nodules is common, increased fibrosis in the nodules and encapsulation never occurs. Swine nodular hepatocellular hyperplasia is classified into two types, nonfibrous and fibrous[20]. The fibrous type possesses some resemblance to the present cases. Specifically, hepatic cords were disordered by fibrous tissue and composed of two or more cells layer. However, stellate scar fibrosis has not been reported in swine. Another type of nodular hyperplastic lesion in domestic animals, designated as *regenerative hepatocellular hyperplasia,* has been reported in dogs[17,21]. Regenerative hepatocellular hyperplasia occurs as a result of chronic hepatic injury, which is hepatic disruption of the normal hepatic parenchymal architecture, and significant fibrosis. In the present cases, fibrosis was found only in the nodules, which were not completely surrounded by fibrous tissue.

Focal nodular hyperplasia occurs in any age group in humans, but many more cases have been reported in women than in men[23,25]. A histopathological feature of focal nodular hyperplasia is central stellate scar with distended vessels[22,23,26]. Bile ducts are usually absent, but a periseptal ductal proliferation may be prominent. Furthermore the hepatocytes are arranged in two-cell-thick plates. The morphological characteristics of the present cases, such as the stellate scar with distended vessels and bile ducts, and the absence of a normal hepatic triad in particular, closely resembled focal nodular hyperplasia in humans[22,23,26], and only one case has been reported in the chimpanzee[3]. It was

considered inappropriate to classify the present cases as regenerative hepatocellular hyperplasia; therefore, they were diagnosed as focal nodular hyperplasia.

The eosinophilic intracytoplasmic inclusions in the hepatocytes, which have not previously been reported in nonhuman primates, were not shown to be viral inclusions by electron microscopical examination. However, the pathogenesis was not identified. Similar homogeneous globular eosinophilic inclusions have been described in the hepatic cytoplasm of untreated laboratory beagle dogs[24].

The pathogenesis of focal nodular hyperplasia in humans is considered to occur by a reactive process following a localized insult to the liver parenchyma; however, this has been controversial[23]. Information on the occurrence of focal nodular hyperplasias that morphologically resemble human cases in readily available experimental animals is valuable.

Acknowledgment: We would like to thank Dr. Shinichiro Nakamura (Shiga University of Medical Science) for his advice and are grateful to Shuichiro Iwashige for his excellent technical assistance.

References

1. Foster JR. Spontaneous and drug-induced hepatic pathology of the laboratory beagle dog, the cynomolgus macaque and the marmoset. Toxicol Pathol. **33**: 63–74. 2005.[Medline] [CrossRef]
2. Ito T, Chatani F, Sasaki S, Ando T, and Miyajima H. Spontaneous lesions in cynomolgus monkeys used in toxicity studies. Exp Anim. **41**: 455–469. 1992.[Medline]
3. Porter BF, Goens SD, Brasky KM, and Hubbard GB. A case report of hepatocellular carcinoma and focal nodular hyperplasia with a myelolipoma in two chimpanzees and a review of spontaneous hepatobiliary tumors in non-human primates. J Med Primatol. **33**: 38–47. 2004.[Medline] [CrossRef]
4. Clark JD, and Olsen RE. Hepatoma in mangabey (*cercocebus artys*). Vet Pathol. **10**: 89–93. 1973.[Medline] [CrossRef]
5. O'Gara RW, and Adamson RH. Spontaneous and induced neoplasms in nonhuman primate. In: Pathology of Simian Primates, part I, TW Fiennes (ed). Karger, Basel. 190–238. 1972.
6. Reindel JF, Walsh KM, Toy KA, and Borowski WF. Spontaneously occurring hepatocellular neoplasia in adolescent cynomolgus monkeys (*Macaca fascicularis*). Vet Pathol. **37**: 656–662. 2000.[Medline] [CrossRef]
7. Yoshizawa K, Oishi Y, Tsubura A, Sano K, Tsubota K, Ikeda K, Fukuhara Y, Senzaki H, and Tsubura A. Hepatocellular carcinoma with PIVKA-II production in a young laboratory monkey. J Toxicol Pathol. **15**: 61–68. 2002. [CrossRef]
8. Tabor E. Nonhuman primate models for non-A, non-B hepatitis. Cancer Detect Prev. **14**: 221–225. 1989.[Medline]
9. Abe K, Kagei N, Teramura Y, and Ejima H. Hepatocellular carcinoma associated with chronic *Schistosoma mansoni* infection in a chimpanzee. J Med Primatol. **22**: 237–239. 1993.[Medline]
10. Borda JT, Ruiz JC, and Sanchez-Negrette M. Spontaneous hepatocellular carcinoma in *Saimiri boliviensis*. Vet Pathol. **33**: 724–726. 1996.[Medline] [CrossRef]
11. Moriss TH, and Abdi MM. Hepatocellular carcinoma in a squirrel monkey (*Saimiri sciureus*). J Med Primatol. **25**: 137–139. 1996.[Medline]
12. Seibold HR, and Wolf RH. Neoplasms and proliferative lesions in 1065 nonhuman primate necropsies. Lab Anim Sci. **23**: 533–539. 1973.[Medline]
13. Brown RJ, O'Neill TP, Kessler MJ, and Andress D. Cholangiocarcinoma in a capuchin monkey (*cebus Albifrons*). Vet Pathol. **17**: 626–629. 1980.[Medline] [CrossRef]
14. Chang J, Wagner JL, and Kornegay RW. Spontaneous cholangiocarcinoma in a ring-tailed lemur (*Lemur catta*). Lab Anim Sci. **29**: 374–376. 1979.[Medline]
15. Kakinuma C, Harada T, Watanabe M, and Shibutani Y. Spontaneous adrenal and hepatic myelolipomas in the common marmoset. Toxicol Pathol. **22**: 440–445. 1994.[Medline] [CrossRef]
16. Beniashvili DS. Tumors of liver, bile duct, and gallbladder. In: Experimental Tumors in Monkeys, Dzhemali Sh. Beniashvili (ed). CRC Press Inc., Boca Raton. 26–42, 1994.
17. Tumors of the Liver. In: Histological of the Alimentary System of Domestic Animals. KW Head, JM Cullen, RR Dubielzig, RW Else, W Misdorp, AK Patnaik, S Tateyama, and I van der Gaag (eds), Second Series, Volume X, Published by the Armed Forces Institute of Pathology in cooperation with the American Registry of Pathology and The World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology, Washington, DC. 121–133. 2003.
18. Bergman JR. Nodular hyperplasia in the liver of the dog: An association with changes in Ito cell population. Vet Pathol. **22**: 427–438. 1985.[Medline]
19. Fabry A, Benjamin SA, and Angelton GM. Nodular hyperplasia of the liver in the beagle dog. Vet Pathol. **19**: 109–119. 1982.[Medline] [CrossRef]
20. Hayashi M, Tsuda H, and Ito N. Histopathological classification of spontaneous hyperplastic liver nodule in slaughtered swine. J Comp Pathol. **93**: 603–612. 1983.[Medline] [CrossRef]
21. Blue JT, French TW, and Meyer DJ. The liver. In: Diagnostic Cytology and Hematology of the Dog and Cat, Second Edition, RL Cowell, RD Tyler, and JH Meinkoth (eds). Mosby, St. Louis. 183–194. 1999.
22. Ishak GK, and Markin SR. Tuomorlike conditions. In: Anderson's Pathology, 10th ed. I Damjanov and J Linder (eds). Mosby, St. Louis. 1832–1839. 1996.
23. Rosai J. Tumors and tumorlike conditions, Liver cell tumors and tumorlike conditions, Focal nodular hyperplasia. In: ROSAI AND ACKERMAN'S SURGICAL PATHOLOGY. 9th ed. J Rosai (ed). Mosby. 992–993. 2004.
24. Greaves P. Liver and Pancreas. In: Histopathology of Preclinical Toxicity Studies, 3rd ed. P Greaves (ed). Elsevier Ltd. 457–569, 2007.
25. Nguyen BN, Fléjou JF, Belghiti TB, and Degott C. Focal nodular hyperplasia of the liver: acomprehensive pathologic study of 305 lesions and recognition of new histologic forms. Am J Surg Pathol. **23**: 1441–1454. 1999.[Medline] [CrossRef]
26. Wanless IR. Vascular disorders. In: Pathology of the Liver. 4th ed. RNM Macsween, AD Burt, BC Portmann, KG Ishak, PJ Scheuer, and PP Anthony (eds). Churchill Livingstone. 573–593. 2002.