Case Report

Two Cases of Rare Hepatocellular Nodular Lesion Caused by Circulatory Disturbance in Rat Livers

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Abstract: Two cases of focal nodular lesions different from regenerative nodules or preneoplasia were found in rat livers. The lesion in Case No. 1 was found in the medial lobe and showed lobular structures with hypertrophic hepatocytes at the center of the lobular structures and atrophic hepatocytes at the periphery. It contained the portal veins but very few bile ducts and hepatic arteries in the central areas. This nodule was diagnosed as nodular regenerative hyperplasia based on its histopathological similarities to such lesions in human cases. The lesion in Case No. 2 was found in the caudate lobe, and contained a large sclerosing portal vein with preserved normal portal triads accompanied by degenerative cellular and inflammatory changes. Based on its histopathological similarities with such lesions in aged canines, this lesion was diagnosed as nodular hyperplasia. Observed vascular abnormalities in both these cases suggested the presence of previous circulatory disturbance with a close anatomical association to the hyperplastic lesions. We, thus, propose a new histopathological entity of hepatocellular hyperplasia which develops in association with local circulatory disturbance and is distinct from post-necrotic regenerative hyperplasia.

Key words: rat, liver, hepatocellular hyperplasia, regeneration, vascular abnormality

Histopathologically, proliferative lesions of hepatocytes in the rat liver are classified into 4 categories: altered hepatocellular foci, regenerative hepatocellular hyperplasia, hepatocellular adenoma and hepatocellular carcinoma. Of these, regenerative hyperplasia has a variety of synonyms such as hepatocellular hyperplasia, focal hyperplasia, multifocal hyperplasia, and nodular hyperplasia. However, the use of the description, regenerative hepatocellular hyperplasia, is limited to lesions of regenerative nodules in the cirrhotic liver. Hepatocellular necrosis has an important role in the pathogenesis of the lesion and its presence is necessary for the diagnosis. In general, nodular lesions are divided into two categories by their distribution: focal (solitary, multiple) or diffuse. While nodular hyperplasia is uncommon as a spontaneously occurring hepatic lesion in rats, regenerative hyperplasia of the diffuse type is noted in association with large granular lymphocyte (LGL) leukemia and sometimes chronic exposure to various hepatotoxins. Solitary hepatocellular nodular lesions as noted above are rare in mice and rats, and no description of these lesions is found in the literature or any textbooks of rodent pathology.

We encountered two rare cases of hepatocellular nodular lesions that could not be classified into any of the 4 categories described above. We propose a new diagnostic terminology for these two nodular lesions based on the histological findings, which suggested the involvement of circulatory disturbance in their pathogenesis.

A female Sprague-Dawley rat (Charles River Laboratories Japan, Inc., Atsugi, Japan), 84 weeks of age, was given physiological saline solution intravenously every day for 26 weeks (Case No. 1). There were no abnormalities in the animal’s general condition or in the clinical laboratory tests conducted at Week 78, the scheduled sacrifice time. A white lesion measuring 12 mm in diameter, showing a slightly lobulated appearance on the cut surface, was found in the median lobe of the liver. No gross abnormalities were noted in the surrounding liver tissue. During gross examination of other organs at necropsy, enlargement of the pituitary gland and a subcutaneous nodule in the genital region were found.

One male Fisher 344/Du rat (Japan SLC Inc., Shizuoka, Japan) from the high-dose group in a 2-year feeding carcinogenicity study (carcinogenicity and hepatotoxicity were negative) was sacrificed at Week 110 (Case No. 2). This animal showed no abnormalities in general condition, body weight or food consumption at any time during the test period. A solitary white nodule (size: 10 × 9 × 7 mm) was
observed in the caudate lobe of the liver. During gross examination of the other organs, atrophy of the thymus, testis and seminal vesicle and white patches in the heart and testis, which are frequently observed in aged rats, were found.

The treatment of both animals had previously been approved by the Animal Care Committee of An-Pyo Center.

The livers of both rats were fixed in 10% aqueous solution of formalin in phosphate-buffered saline after necropsy. After fixation, the liver was trimmed, processed routinely, and embedded in paraffin. Thin sections obtained from the paraffin block (3 µm in thickness) were stained with hematoxylin and eosin (HE) and by several special staining methods as follows: Periodic-acid Schiff (PAS), Schmorl’s stain, Perl’s Prussian blue stain, Masson’s trichrome stain and Watanabe’s silver impregnation stain. In addition, immunohistochemical staining was also performed by a labeled streptavidin-biotin method with several antibodies, such as anti-porcine vimentin antibody (Dako A/S, Glostrup, Denmark), anti-human desmin antibody (Dako A/S), anti-human α-smooth muscle actin (α-SMA) antibody (Dako A/S), anti-human von Willebrand factor (vWF) antibody (Dako A/S), anti-rat macrophage antibody (clone: ED-1, Serotec Ltd., Oxford, England) and anti-rat proliferating cell nuclear antigen (PCNA) antibody (Dako A/S) as the primary antibodies. Pretreatment was conducted by microwave oven heating for vimentin and PCNA, trypsin digestion for vWF and pepsin digestion for ED-1. Immunoreaction with the primary antibodies was allowed to occur for 12 hours at 4°C. Thereafter, biotinylated anti-rabbit or mouse IgG antibody and peroxidase-labelled streptavidin were applied to the sections for 10 min each at room temperature. The reaction was visualized with 3,3′-diaminobenzidine in 0.1 M tris HCl buffer (pH 7.6) plus hydrogen peroxide. Sections were washed with TBS-T (Tris buffer solution plus tween 20) at each step. Finally, all the sections were counterstained with Mayer’s hematoxylin. Desmin was used as a marker for the hepatic stellate cells (Ito cells), but no appropriate immunoreactivity could be obtained, possibly because of excessive fixation by formalin. Therefore, vimentin-positive, vacuoles-containing cells in the sinusoids were considered as stellate cells.

In addition, for Case No. 1, serial sections of the liver portion containing the nodule and of the surrounding liver tissue at the porta hepatis were prepared to detect vascular abnormalities suggestive of the pathogenesis. All sections were examined under a light microscope.

In Case No. 1, histopathologically, the nodular lesion (Fig. 1) consisted of uniform lobular structures with centrally located venous vessels (Fig. 2). The normal radial orientation of the hepatic plates was present, however, no portal triads were found. Regularly arranged plates of hypertrophic hepatocytes were seen around the center and atrophic hepatocytes were found at periphery of the lobular structure, accompanied by fatty degeneration (Fig. 3) or single-cell or focal necrosis with inflammatory cell infiltration and/or condensation of reticulin fibers. Although the border between the nodule and the surrounding liver tissue was distinct, compression of the surrounding liver tissue by the nodule was not obvious. The hepatocytes in the

![Fig. 1. Cut surface showing the whole appearance of the nodule (Case No. 1), ×2. HE stain.](image)

![Fig. 2. The nodule consists of regularly arranged lobular structures with regular sizes and centrally locating venous vessels (arrows), ×40. HE stain.](image)

![Fig. 3. The lobular structure consists of hypertrophic hepatocytes in the central area and atrophic hepatocytes showing fatty degeneration (arrows) in the peripheral area, ×200. HE stain.](image)
A nodule had nuclei with scant chromatin and one or two conspicuous nucleoli and showed a diffuse basophilic cytoplasm, with no cellular atypia or mitoses. In addition, some hepatic cords were multi-layered (Fig. 3) and multinucleated hepatocytes were sometimes observed, especially at the peripheral portion of the lobular structures. The degree of hemosiderin deposition in the sinusoidal lining cells was more prominent in the surrounding liver tissue than in the nodule. No lipofuscin deposition or glycogen storage was observed in the hepatocytes in either the nodule or the surrounding liver tissue.

Examination of serial tissue sections demonstrated that the venous radials located at the center of the lobular structure had the characteristics of portal vein tributaries, and were occasionally associated with small bile ducts (Fig. 4a) or proliferating bile ductules, but rarely hepatic arteries (Fig. 4b), principally in the vicinity of the portal vein. Interestingly, larger portal tracts without interlobular arteries were also observed adjacent to the nodule (Fig. 5a) and at the porta hepatis. There were scarcely any arterial components

**Fig. 4a.** Bile ductular components (arrows) around the vessel located in the center of the lobular structure, ×328. HE stain.

**Fig. 4b.** Complete portal triads are extremely rare in the center areas (*) of the lobular structure. Arrow indicates a possible interlobular artery, ×328. HE stain.

**Fig. 5a.** A large portal area adjacent to the nodule (arrows). Asterisk indicates venous vessel, not accompanying arterial components, ×30. HE stain.

**Fig. 5b.** Venous vessel (*) branching off in several directions into the nodule (arrows) is seen. This vessel connects to the large portal area (open star) seen in Fig. 5a. ×60. HE stain.

**Fig. 5c.** Higher magnification of Fig. 5a (open square) showing bile ducts (arrows) and pigment-laden macrophages (arrowheads) in the vessel wall and apoptotic necroses of hepatocytes (open star) in the vicinity of the vessel wall, ×328. HE stain.
in the nodule. The larger portal tracts around the nodule were connected to venous vessels which showed branching in several directions into the nodule (Fig. 5b). They also showed accumulation of hemosiderin-laden macrophages, hemorrhage and single cell necrosis of the surrounding hepatocytes (Fig. 5c). No fibrosis in the nodule or encapsulation around the nodule was observed.

Immunohistochemical studies revealed the presence of fewer ED-1-positive cells in the nodules than in the surrounding liver tissue (Fig. 6), and no vWF immunoreactivity was detected in the nodule, except in the venous vessels. Alpha-SMA-positive cells were observed in dilated sinusoids and vessel walls. No GST-P-positive hepatocytes were detected in any region of the liver tissue, including the nodule. The number of stellate cells and PCNA-positive hepatocytes in the nodule were comparable to those in the surrounding liver tissues, but the stellate cells were accumulated in the vicinity of hepatocytes showing fatty degeneration.

In Case No. 2, the nodule (Fig. 7) showed a normally preserved lobular architecture with portal triads (Fig. 8), but the normal radial orientation of the hepatic cell cords around the central vein was lost. In addition, the nodule clearly compressed the surrounding liver tissue. An unusually large portal area was observed in the caudate lobe, and it contained a sclerosing portal vein (Fig. 9a). The nodule showed prominent microgranuloma formation, neutrophil accumulation and accumulation of lipofuscin- or hemosiderin-laden foamy cells (Fig. 9b). Most of the hepatocytes within the nodule were swollen and showed microvesicular fatty degeneration, glycogen storage (Fig. 9b) and single-cell or focal necrosis. Although rare, the hepatocytes within the nodule showed mitoses and were multi-layered. Fibrosis was rarely observed within the nodule and was not found at all in the surrounding liver tissue.

The expression of neither α-SMA nor vWF was observed in the sinusoids within the nodule. The number of PCNA-positive hepatocytes was slightly increased in the nodule as compared with that in the surrounding liver tissue. ED-1-positive cells were evident in the nodule and were larger in size than those in the surrounding liver tissue. Accumulation of many stellate cells was observed around hepatocytes showing fatty degeneration. GST-P-positive hepatocellular foci were observed in the nodule, but not in the surrounding liver tissue.

The results of immunohistochemical analysis of both cases No. 1 and No. 2 are summarized in Table 1. We consider the lesions in both Case No. 1 and Case No. 2 represented regenerative hyperplasia of the hepatocytes, because both nodules showed partially preserved normal portal triads and contained multi-layered hepatocytes. Also, the hepatocytes within the nodules showed no evidence of cellular atypia.

In Case No. 1, a regular lobular architecture consisting of hypertrophic hepatocytes in the central areas and atrophic hepatocytes in the peripheral regions was characteristic, and
it was readily distinguishable from hepatocellular tumors and altered hepatocellular foci, which can be induced experimentally in rats by porta-caval anastomoses or repeated oral administration of selenium, and can also develop spontaneously in rats bearing LGL leukemia. In human beings, such lesions would be diagnosed as nodular regenerative hyperplasia (NRH) because of a non-uniform blood supply, and unusual blood supply is considered to play an important role in their pathogenesis. In the present case, unusual blood supply, as evidenced by a lack of arteries in the large portal area, may have been related to the pathogenesis. Moreover, hemosiderin deposition in the vessel walls suggested the presence of a circulatory disturbance causing repeated hemorrhage. Human NRH is known to characteristically exhibit obliteration of the small portal veins, and may be predominantly supplied by arterial blood. However, the main vessels supplying nutrients in the present case appeared to be the portal vein. Fully developed and typical bile ducts were quite rare adjacent to the portal veins within the nodule in Case No. 1. Bile ducts in human NRH are also usually known to be atrophic or disappearing.

Use of the description, regenerative hepatocellular hyperplasia, in rodents is recommended for regenerative nodules as seen in liver cirrhosis or in the liver of animals with LGL leukemia, with associated evident hepatocellular necrosis as a necessary criterion. Although hepatocellular necrosis was not evident in our present cases, the diagnosis of focal NRH was considered to be appropriate because of the morphological similarity to NRH as seen in human cases. In addition, the histological characteristics were different from those of diffuse-type NRH in rats.

The major histopathological characteristic in Case No. 2 was the preserved normal portal triads. In addition, degenerative changes in hepatocytes and inflammatory changes were also observed. These findings in Case No. 2 were consistent with the morphological features of nodular hyperplasia in the liver of canines: that is, compression, thickened plates, presence of portal areas and well-differentiated hepatocytes with accumulation of lipid and glycogen, and lipogranuloma formation. In domestic animals, nodular hyperplasia develops in older dogs but is rare in other species. Nodular hyperplasia of the liver in

<table>
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<th>Case No.1 Perinodular area</th>
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<td>Hepatocytes</td>
<td>PCNA –</td>
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<td>Sinusoidal lining cells</td>
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<td>Macrophage (ED-1)</td>
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Gradings: –; negative, ±; minimal, +; slight, ++; moderate.
a) limited to area showing a sinusoidal dilatation, b) clone name.

Table 1. Comparison of Antigen Expression in the Cell Components Between Nodule and Perinodular Areas in the Liver

Fig. 9a. Higher magnification of Fig. 8. Portal area in the vicinity of the nodule contains the portal vein (arrows) showing a sclerotic change. The compression by the nodule of the surrounding liver tissue is evident (arrowheads), ×140. HE stain.

Fig. 9b. Higher magnification of Fig. 8. The hepatocytes consisting of the nodule show hypertrophy, but sinusoids are unclear. Microgranuloma formation (arrowheads) and accumulation of pigment-laden macrophages (small arrows) are frequently observed. Large arrow indicates a normal portal triad, ×220. HE stain.
pigs has been reported in young, slaughtered animals (of age 6 months). In contrast, the normal porcine liver shows well-developed interlobular connective tissues and the characteristic portal-portal bridging, with numerous capillary vessels in the fibrous bands. These species-specific anatomical structures may have some bearing on the causes of nodular hyperplasia in the liver of swine, even in young animals.

In humans, there is a specific entity called focal nodular hyperplasia (FNH), which is characterized by the loss of normal liver architecture, existence of a central scar and morphologically abnormal vessels. This lesion may also be the result of abnormal blood flow due to anomalous arteries or angiogenesis as seen in NRH. The nodule in Case No. 2 was found to be supplied by an abnormal vessel showing a sclerotic change in the large portal triads at the base of the caudate lobe. FNH in humans is supplied by arterial blood and shows sinusoidal capillarization. Although Case No. 2 showed no abnormalities in the arterial components or sinusoidal capillarization, it was also probably mainly supplied by portal veins as seen in Case No. 1.

Interestingly, GST-P-positive hepatocellular foci were limited to the nodule in Case No. 2. Although Case No. 2 also showed glycogen storage in the hepatocytes within the nodule, known to be the first cytopathological change developing in hepatocytes after treatment with hepatocarcinogens, the significance of the GST-P expression, a marker for preneoplastic lesions in the liver, in Case No. 2 was not elucidated.

For non-neoplastic hepatocellular nodular lesions in humans, several terminologies, including nodular regenerative hyperplasia, partial nodular transformation, idiopathic portal hypertension, focal nodular hyperplasia and regenerative nodules, have been used. These lesions, except for the regenerative nodules, predominantly result from vascular abnormalities and show an apparent heterogeneity of the blood supply or morphological abnormalities in the portal tracts as the common factors in their pathogenesis. Consequently, these lesions may be classified into the same category. Namely, the location and distribution of the abnormalities in the portal tracts may affect the size or distribution of the nodules in the liver. The hyperplastic lesions reported in the present study might have developed as a result of weak but persistent liver cell necrosis and/or degeneration due to mild circulatory disturbance, different from massive liver cell necrosis due to rapid circulatory disturbance or direct injury by toxic or infectious agents.

In the sinusoids of hepatocellular tumors in humans and dogs, several phenomena such as capillarization, appearance of α-SMA-positive cells, and a decrease in the number of Kupffer cells and stellate cells as compared with the normal liver have been reported. Therefore, we focused on the sinusoids in both of our present cases to clarify the nature of the sinusoidal lining cells. Immunostaining of the nodules revealed no expression of vWF in the sinusoidal endothelial cells, diminution/abundance of ED-1-positive cells, or vacuole-containing stellate cells in the sinusoids in either case. These results are not consistent with observations in experimental rats showing hepatocarcinogenesis, which show vWF expression in the sinusoidal endothelial cells, loss of Kupffer cells and diminution of lipid droplets in the stellate cells. VWF expression in the sinusoidal endothelial cells suggests the dominance of arterial blood supply rather than neoplastic properties, because sinusoidal capillarization in human FNH is known to have a blood supply mainly from arteries.

In conclusion, we presented here two cases of hepatocellular hyperplasia that developed in relation to circulatory disturbance as evidenced by concurrent vascular abnormalities in rats. This type of hyperplastic lesion should be classified into a new histopathological entity that can be distinguished from regenerative hyperplasia secondary to massive liver cell necrosis either by rapid circulatory disturbance or direct injury by toxic or infectious agents.

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References