

Case Report

## Hepatocellular adenoma with severe fatty change in a male Spontaneously Diabetic Torii rat

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**Abstract:** The Spontaneously Diabetic Torii (SDT) rat is a rat model of nonobese type 2 diabetes mellitus, and hepatocellular adenomas have not been reported in this model. We report a hepatocellular adenoma with severe fatty change in a male 42-week-old SDT rat fed a high-fat diet. At necropsy, the animal had a whitish nodular mass of approximately 2 cm in diameter in the right medial lobe. Histologically, the mass was well demarcated from the surrounding tissues, slightly compressing the adjacent hepatic parenchyma and widely compartmented by fibrous connective tissues. The mass consisted of vacuolated tumor cells resembling hepatocytes with a solid and occasionally trabecular growth pattern. Abundant neutral lipids, which were positive for fat with Oil Red O stain and which ultra-structurally had moderately dense material, were contained within the vacuoles of the tumor cells. Immunohistochemically, the tumor cells showed an increase in immunoreactivity or number for Cytokeratin 8/18 and proliferating cell nuclear antigen but were negative for mesenchymal markers. From these findings, the mass could be distinguished from hepatocellular hyperplasia and was diagnosed as hepatocellular adenoma. In rats, hepatocellular adenoma accompanied by severe fatty change is rare, and this is the first report of a hepatocellular tumor with severe fatty change in a SDT rat. (DOI: 10.1293/tox.2016-0051; J Toxicol Pathol 2017; 30: 69–73)

**Key words:** hepatocellular adenoma, fatty change, liver, Spontaneously Diabetic Torii rat, microscopic finding

The Spontaneously Diabetic Torii (SDT) rat is known as a nonobese type 2 diabetes rat model that achieves diabetic status with high plasma glucose levels by 42 weeks of age<sup>1</sup>. However, hepatocellular tumors have not been reported in the SDT rats at that age. This case report describes a rare hepatocellular adenoma in a 42-week-old male SDT rat that was fed a high-fat diet.

Five male SDT rats (five weeks of age) were purchased from CLEA Japan, Inc. (Tokyo, Japan). The treatment and handling of the animals were approved by the committee for the humane care and use of animals of Biological/Pharmacological Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc. All animals were housed in a controlled room (temperature of 23 ± 3°C, humidity 55 ± 15%, and 12 hr lighting cycle) and were allowed free access to diet and water. The animals were fed a standard commercial diet (CRF-1, Oriental Yeast Co., Ltd.,

Tokyo, Japan) up to 35 weeks of age. From 36 weeks of age, they were fed a commercial high-fat diet (D09100301, Research Diets, Inc., New Brunswick, NJ, USA) for 6 weeks until 42 weeks of age. All the animals showed diabetic hyperglycemia until the day before euthanasia but did not show any remarkable changes except for those of diabetes.

In one animal at necropsy, the liver was whitish yellow in color and contained a whitish nodular mass of approximately 2 cm in diameter in the right medial hepatic lobe (Fig. 1). The cut surface after formalin fixation consisted mainly of a whitish parenchyma and contained some multiple cystic spaces. There were no macroscopically similar findings in the other rats. An additional experiment with 9 male SDT rats was conducted under the same conditions, but no hepatocellular lesions were observed in any other rat.

The mass was fixed in 10% neutral-buffered formalin, embedded in paraffin wax, and sectioned. Specimens were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), Oil Red O, and Sirius Red. Immunohistochemistry was performed with antibodies against proliferating cell nuclear antigen (PCNA) (Clone PC10, 1:500), vimentin (Clone V9, 1:100), desmin (Clone D33, 1:100), and  $\alpha$ -smooth muscle actin (SMA) (Clone 1A4, 1:50) purchased from Dako A/S, Glostrup, Denmark, Cytokeratin 8/18 (CK 8/18) (1:600, Progen Biotechnik GmbH, Heidelberg, Germany), glutathione

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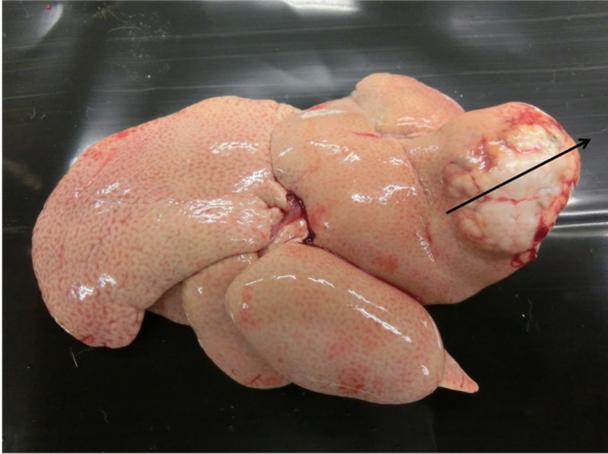
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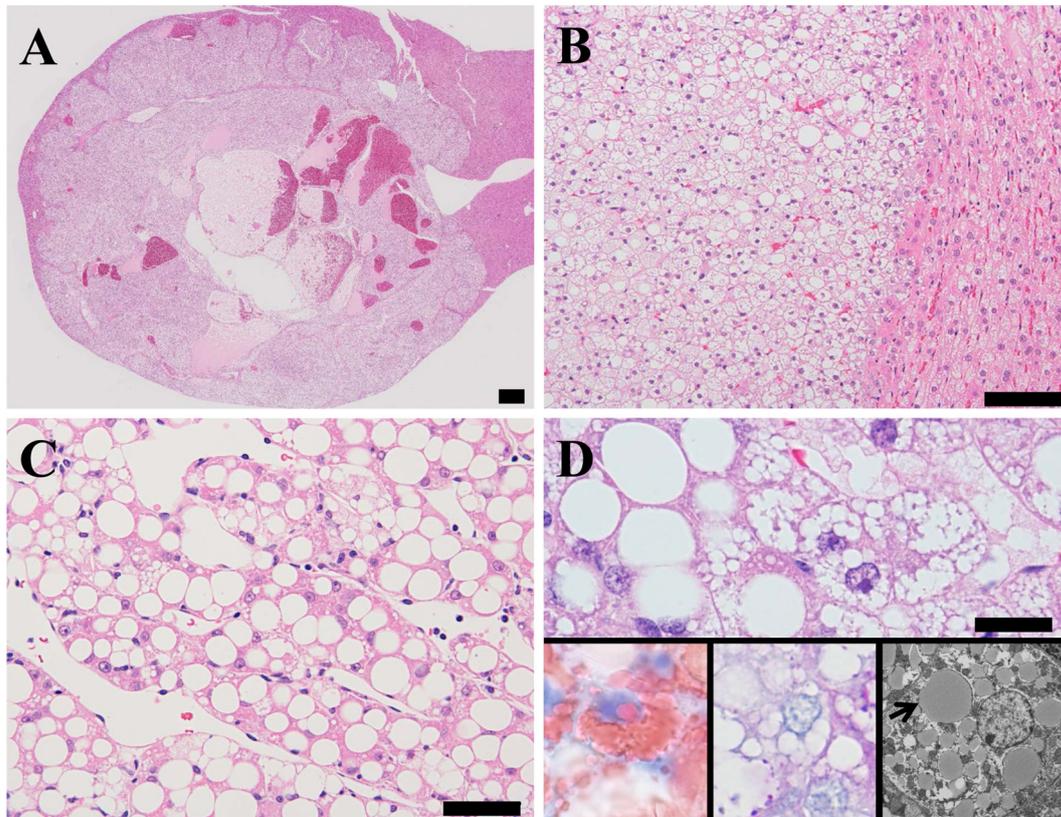
S-transferase placental form (GST-P) (1:1000, MBL Co., Ltd., Nagoya, Japan), and CD68 (Clone ED-1, 1:100, BMA Biomedicals, Augst, Switzerland) using Histofine Simple



**Fig. 1.** A whitish nodular mass of approximately 2 cm in diameter was located in the right medial hepatic lobe. The mass was cut along the direction of the arrow.

Stain MAX-PO (Nichirei, Tokyo, Japan). Sections were also subjected to a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay using an ApopTag® Peroxidase *In Situ* Apoptosis Detection Kit (Millipore, Billerica, MA, USA). For electron microscopy, parts of the formalin-fixed tissue specimens were postfixed in 2.5% glutaraldehyde and phosphate buffered 2% osmic acid. Postfixed specimens were embedded in resin in accordance with the Quetol embedding method. Resin-embedded semi-thin sections stained with toluidine blue were prepared and were examined microscopically in order to determine the areas for ultrathin sectioning. Ultrathin sections were stained with uranyl acetate and lead acetate and were examined under a transmission electron microscope (HT-7700 electron microscope, Hitachi High-Technologies Corp., Tokyo, Japan).

Histologically, the nodular lesion was well demarcated from the slightly compressed adjacent hepatic parenchyma and widely compartmentalized by fibrous connective tissues (Fig. 2A). The mass consisted mainly of a solid growth pattern of hepatocyte-like cells in the entire area of the lesion and occasionally a trabecular growth pattern with two to several layers in the center of the lesion (Fig. 2B and 2C).



**Fig. 2.** A) The nodular lesion was well demarcated from surrounding slightly compressed adjacent hepatic parenchyma and widely compartmentalized by fibrous connective tissues. B) A solid growth pattern of vacuolated hepatocyte-like cells was observed in the entire area of the mass. C) A trabecular growth pattern with two to several layers of hepatocyte-like cells was observed in the center of the mass. D) The hepatocyte-like cells contained large to small vacuoles that were positive with Oil Red O (lower left inset) and negative on PAS staining (lower middle inset). Moderately dense figures (arrow) were observed in the hepatocyte-like cells by transmission electron microscopy (lower right inset). Bars: 1 mm (A), 100  $\mu$ m (B), 50  $\mu$ m (C), 20  $\mu$ m (D).

The normal lobular architecture had almost completely disappeared, and the portal triads and central veins were not clearly visualized. The hepatocyte-like cells contained large to small vacuoles (Fig. 2D). These cells were also sporadically accompanied by nuclear vacuoles. These vacuoles were positive with Oil Red O and negative with PAS staining and contained moderately dense material on transmission electron microscopy. Mitotic figures were observed to be slightly scattered, and there were more than in the surrounding parenchyma. Macrovesicular cells and microvesicular circular/short spindle cells were observed around the fibrous connective tissues, which were positive on Sirius Red, within the mass. Multiple cystic structures containing blood and cell debris were present in the center of the mass. Slight lymphocytic infiltration was scattered throughout the mass.

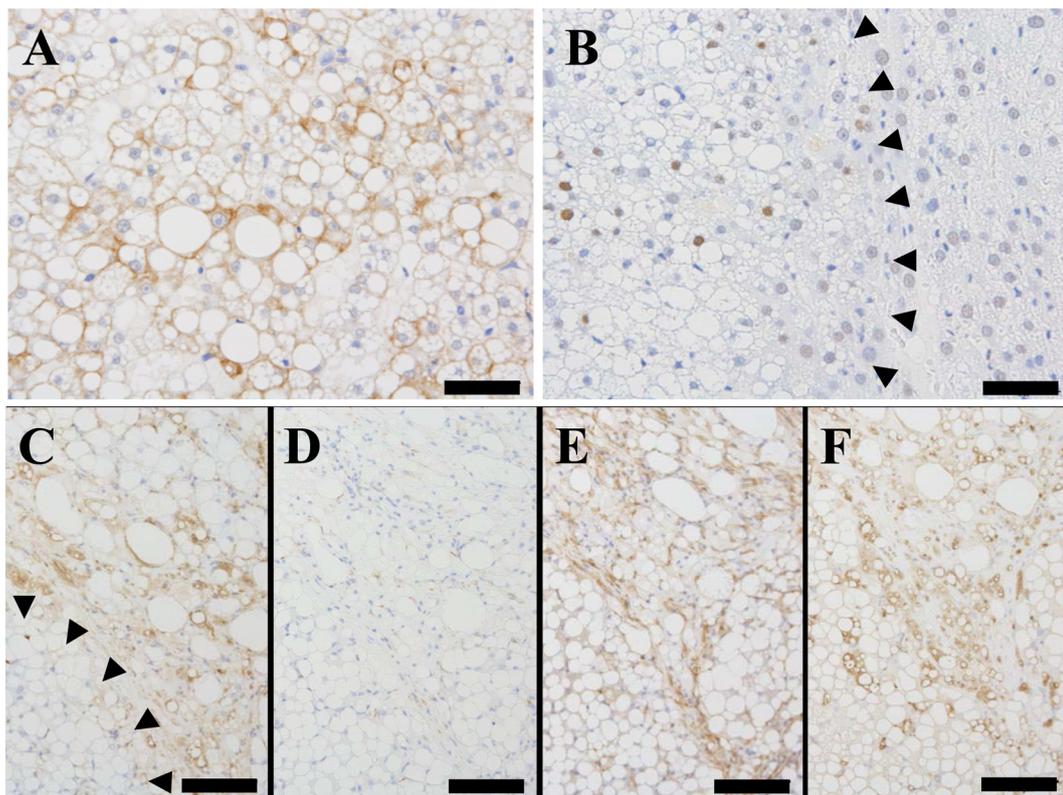
The results of immunohistochemical staining are summarized in Table 1. Hepatocyte-like cells showed an increase in the intensity or numbers of cells staining for CK 8/18 and PCNA but were negative for GST-P, vimentin, desmin,  $\alpha$ -SMA, and CD68 (Fig. 3). There were no marked differences in the growth patterns and localization. On the

**Table 1.** Results of Immunohistochemical Examination and TUNEL Assay

Markers	Nodular area		Surrounding nodular area
	Hepatocyte-like cells	Macrovesicular/microvesicular cells	Hepatocytes
CK 8/18	+*	-/-	±
PCNA	+**	NE	±
GST-P	-	+/-	-
Vimentin	-	+/+	-
Desmin	-	-/+	-
$\alpha$ -SMA	-	-/+	-
CD68	-	+/-	-
TUNEL	-	NE	-

NE: Not examined. Criteria for grading: -, negative; ±, weakly positive/slightly increasing positive cells; +, positive/increasing positive cells.

\*Increasing positive intensity and numbers in the nodular area as compared with surrounding area. \*\* Increasing positive cells in the nodular area as compared with surrounding area.



**Fig. 3.** A) Most of the hepatocyte-like cells showed increased positive intensities and numbers for CK8/18. B) PCNA-positive cells were increased in the nodular mass, indicated by arrowheads, compared with the surrounding hepatic cells. C)–F) were taken from same location. The mass indicated by arrowheads in C) showed a solid pattern of hepatocyte-like cells. The mass showed fibrous connective tissue within the mass. C) Vimentin was negative in the hepatocyte-like cells but positive in the macro/microvesicular cells adjacent to the fibrous connective tissue. D) Desmin was negative in the hepatocyte-like cells but positive in the microvesicular cells adjacent to the fibrous connective tissue. E)  $\alpha$ -SMA was negative in the hepatocyte-like cells but positive in the microvesicular cells adjacent to the fibrous connective tissue. F) CD68 was negative in the hepatocyte-like cells but positive in the macrovesicular cells adjacent to the fibrous connective tissue. Bars: 50  $\mu$ m (A and B), 100  $\mu$ m (C–F).

other hand, macro/microvesicular cells around the fibrous connective tissues were positive for vimentin, desmin,  $\alpha$ -SMA, or CD68. TUNEL-positive apoptotic cells were not observed in the mass.

The hepatic nodular mass in the present case was considered to be derived from hepatocytes because the majority of the cells comprising the mass resembled hepatocytes in particular with a rounded nucleus. In addition, cells were observed with increased positive intensities and numbers for CK 8/18, which has been reported to be detected in hepatocellular preneoplastic lesions and neoplastic lesions<sup>2</sup>. Small to large vacuoles in these cells were revealed to be neutral lipids based on their positive reaction with Oil Red O staining and their ultrastructural findings. Furthermore, these cells showed increased positive intensities or numbers for PCNA, which is a well-known marker for proliferating cells, and CK8/18. These findings suggested that this lesion was a hepatocellular proliferative lesion. According to the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND), hepatocellular proliferative lesions are classified as nonneoplastic lesions (focus of cellular alteration, hyperplasia) and neoplastic lesions (adenoma and carcinoma)<sup>3</sup>. GST-P is an established marker for preneoplastic and neoplastic lesions induced by hepatocarcinogens, but some hepatocarcinogens are known to cause lesions that are negative for GST-P<sup>4</sup>. Although the lesion in this case was negative for GST-P, the solid proliferative hepatocytes, slight compression of the adjacent tissue, and macroscopically large size were consistent with criteria for adenoma<sup>3</sup>. Therefore, the mass was diagnosed as a "hepatocellular adenoma."

Differential diagnosis of hepatocellular foci of cellular alteration, hyperplasia, and carcinoma is discussed below. A hepatocellular focus of cellular alteration is less than several lobules in diameter, with minimal compression, and also maintains the lobular architecture<sup>3</sup>. Hepatocellular hyperplasia of the non-regenerative/regenerative type slightly or moderately compresses the adjacent tissues following hepatocellular proliferation and results in a size of several hepatic lobules or several millimeters in diameter. It also maintains the lobular architecture and is sometimes accompanied by fibrous connective tissue. Angiectasis is occasionally shown in non-regenerative hepatocellular hyperplasia. Hepatocellular carcinoma shows a lack of distinct demarcation, proliferation of pleomorphic tumor cells, and loss of the normal lobular architecture. On the other hand, hepatocellular adenoma ranges in size from one millimeter to be visually recognizable with loss of the normal lobular architecture and an irregular growth pattern. In the present case, the bile ducts and the fibrous tissues within the mass had hepatocellular hyperplastic characteristics. However, the bile ducts remaining within a hepatocellular adenoma was reported in the mouse<sup>5</sup>. It was speculated that the fibrous tissues surrounding the mass were involved in the mass due to hepatocellular proliferation. While it is known that there is no formation of trabecular layers in hepatocellular hyperplasia. Additionally, the large size of the lesion

exceeded that seen for hyperplasia, and the distinct demarcation seen in this case is not consistent with carcinoma<sup>3</sup>. Therefore, this mass was diagnosed as a hepatocellular adenoma. Additionally, there were more lipid vacuoles in the tumor cells than in surrounding tissue. In terms of clinical chemistry, plasma total cholesterol and triglyceride levels were not increased in this animal when compared with the other animals during the high-fat diet feeding period (data not shown). A hepatocellular adenoma in a diet-induced obese mouse has histological characteristics similar to those of the present case, such as cell proliferation with prominent fatty change, which was considered to be affected by the high-fat diet<sup>5</sup>. It was speculated that incorporation of lipids from the high-fat diet into tumor cells was promoted. In the case of humans, highly differentiated hepatocellular carcinoma is sometimes accompanied by fatty change<sup>6</sup>. Severe fatty change was a characteristic of this tumor, so the lesion was diagnosed as hepatocellular adenoma with severe fatty change. Furthermore, there were macrovesicular/microvesicular cells adjacent to the fibrous connective tissues within the mass. Macrovesicular cells were considered to be macrophages because they were positive for vimentin and CD68. Microvesicular cells were considered to be derived from Ito cells because they were positive for vimentin, desmin, and  $\alpha$ -SMA and showed a circular/short spindle morphology, which resembled Ito cells and myofibroblasts derived from Ito cells. Interestingly, macrovesicular cells were also positive for GST-P in the present case. A previous study reported that GST-P was found in bile-duct epithelial cells and a few hepatocytes in the rat liver<sup>7</sup>; however, it was not clear whether macrovesicular cells considered to be macrophages were positive for GST-P. Considering that the tumor was only observed in 1 out of 14 cases under the same conditions, the present case was likely spontaneous. To our knowledge, this is the first case report of spontaneous hepatocellular adenoma with severe fatty change presenting in an SDT rat.

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**Disclosure of Potential Conflicts of Interest:** The authors declare that they have no conflicts of interest to disclose in connection with this report.

## References

1. Shinohara M, Masuyama T, Shoda T, Takahashi T, Katsuda Y, Komeda K, Kuroki M, Kakehashi A, and Kanazawa Y. A new spontaneously diabetic non-obese Torii rat strain with severe ocular complications. *Int J Exp Diabetes Res*. 1: 89–100. 2000. [Medline] [CrossRef]
2. Kakehashi A, Inoue M, Wei M, Fukushima S, and Wanibuchi H. Cytokeratin 8/18 overexpression and complex formation as an indicator of GST-P positive foci transformation into hepatocellular carcinomas. *Toxicol Appl Pharmacol*.

- 238**: 71–79. 2009. [[Medline](#)] [[CrossRef](#)]
3. Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, Malarkey DE, Kaufmann W, Küttler K, Deschl U, Nakae D, Gregson R, Vinlove MP, Brix AE, Singh B, Belpoggi F, and Ward JM. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol Pathol.* **38**(Suppl): 5S–81S. 2010. [[Medline](#)] [[CrossRef](#)]
  4. Sakai M, and Muramatsu M. Regulation of glutathione transferase P: a tumor marker of hepatocarcinogenesis. *Biochem Biophys Res Commun.* **357**: 575–578. 2007. [[Medline](#)] [[CrossRef](#)]
  5. Kawai K, Sakairi T, Tanaka M, Shinozuka J, Ide M, Sato H, Fujiwara T, Sano F, and Kume E. A hepatocellular adenoma in a diet-induced obese mouse. *J Toxicol Pathol.* **23**: 59–62. 2010. [[Medline](#)] [[CrossRef](#)]
  6. Hirohashi S, Ishak KG, Kojiro M, Wanless IR, Theise ND, Tsukuma H, Blum HE, Deugnier Y, Puig PL, Fischer HP, and Sakamoto M. Hepatocellular carcinoma. In: World Health Organization (WHO) classification tumours of the digestive system. Hamilton SR, Aaltonen LA (eds). IARC Press, Lyon. 158–172. 2000.
  7. Tee LB, Gilmore KS, Meyer DJ, Ketterer B, Vandenberghe Y, and Yeoh GC. Expression of glutathione *S*-transferase during rat liver development. *Biochem J.* **282**: 209–218. 1992. [[Medline](#)] [[CrossRef](#)]