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Case Report

Cystic Cholangioma in the Thoracic Cavity of a Rat

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Abstract: A female congenic rat produced by repeated backcrossing of Nihon rats, a model for hereditary renal cell carcinoma, to Brown Norway rats was necropsied at 24 months of age. At necropsy, a white mass about 1 centimeter in size was observed in the thoracic cavity, and the mass partly adhered to the esophagus and the diaphragm. Histologically, the mass was clearly circumscribed by connective tissue, and consisted of neoplastic cuboidal epithelial cells that showed cystic tubular proliferation. Some islands of well-differentiated hepatocytes and some vessels were observed in the mass. Immunohistochemically, the tumor cells were strongly positive for cytokeratin and partly positive for vimentin but were negative for mesothelin and Von Willebrand Factor. The positive rate for Ki-67 was 2.4%. Based on these histological and immunohistochemical evidences, we diagnosed this tumor as a cystic cholangioma that might have arisen from the ectopic hepatic tissue in the thoracic cavity. (DOI: 10.1293/tox.25.41; J Toxicol Pathol 2012; 25: 41–44)

Key words: cystic cholangioma, rat, thoracic, diaphragmatic hernia, congenital, ectopic liver

Cholangioma is a rare benign tumor of control rodents and is divided into two types: simple and cystic[1–3]. In general, cystic cholangioma is a uniform tumor that is well-circumscribed and expansile, often compressing adjacent parenchyma. The gross appearance of this tumor varies from firm, grey-white nodules to lesions with a spongy texture. Histologically, cystic cholangioma forms unilocular or multilocular cysts lined by single-layered flat or cuboidal epithelia. Recently, we encountered a case of a cystic cholangioma in the thoracic cavity of a rat. The tumor in this case was considered to have arisen from the ectopic hepatic tissue in the thoracic cavity. In this paper, we describe the histological and immunohistochemical features of this rare case.

A female congenic rat was produced by repeated backcrossing of Nihon rats, a model for hereditary renal cell carcinoma[3], to Brown Norway rats and was maintained in a barrier facility until 24 months of age. The procedures for animal care and housing followed accredited in-house animal welfare principles. At 24 months of age, the animal was euthanized by exsanguination under sodium pentobarbital anesthesia and necropsied. At necropsy, a white mass about 1 centimeter in size was observed in the thoracic cavity, and the mass partly adhered to the esophagus and the diaphragm (Fig. 1). No abnormality was observed in the lungs and the heart. In the abdominal cavity, a part of the liver adhered to the diaphragm, and the membrane in the adhesive area was markedly thickened. After necropsy, all organs including the white mass were fixed in neutral buffered 10% formalin. The white mass, the esophagus, the diaphragm and the liver were embedded in paraffin wax, sectioned at 3 microns and stained with hematoxylin and eosin (HE). For immunohistochemical examination, the sections of the white mass were subjected to a labeled polymer method using Histofine Simple Stain Rat MAX-PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan) for antibodies against cytokeratin (N1590, predilution, Dako, Carpinteria, CA, USA), CK20 (N1627, predilution Dako), vimentin (N1521, predilution, Dako), albumin (A0001, 1:500, Dako), mesothelin (28001, 1:250, IBL, Gunma, Japan), Von Willebrand Factor (N1505, 1:500, Dako) and Ki-67 (M7248, 1:75, Dako), then the sections were counterstained with hematoxylin. The percentage of Ki-67-positive tumor cells in 10 high-power fields was calculated.

Histologically, the white mass was clearly circumscribed by connective tissue (Fig. 2A) and consisted of neoplastic cuboidal or flattened epithelial cells that showed cystic tubular proliferation (Fig. 2B). The tumor cells showed low-grade atypia, and mitotic figures were rare. Some islands of well-differentiated hepatocyte-like cells and some vessels were observed in the mass (Fig. 2C). A portal triad was not observed in the islands. In the adhesion area be-

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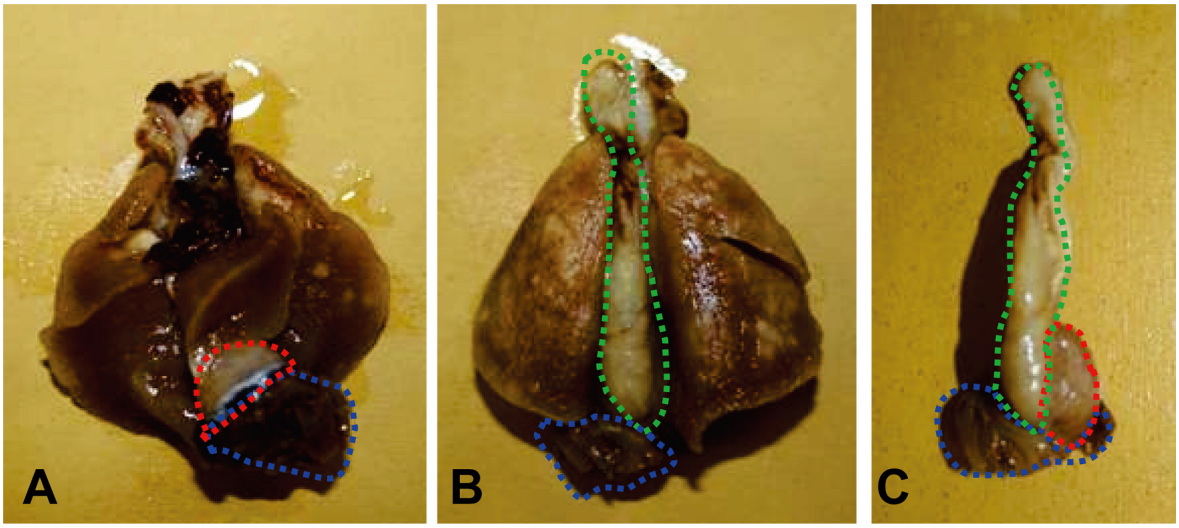

Fig. 1. Gross features of the white mass after fixation in neutral buffered 10% formalin. A: The costal surface of the lungs. B: The internal surface of the lungs. C: After removal of the lungs. Red, blue and green dotted lines encircle the white mass, the diaphragm and the esophagus respectively. The white mass adhered to the esophagus and the diaphragm.

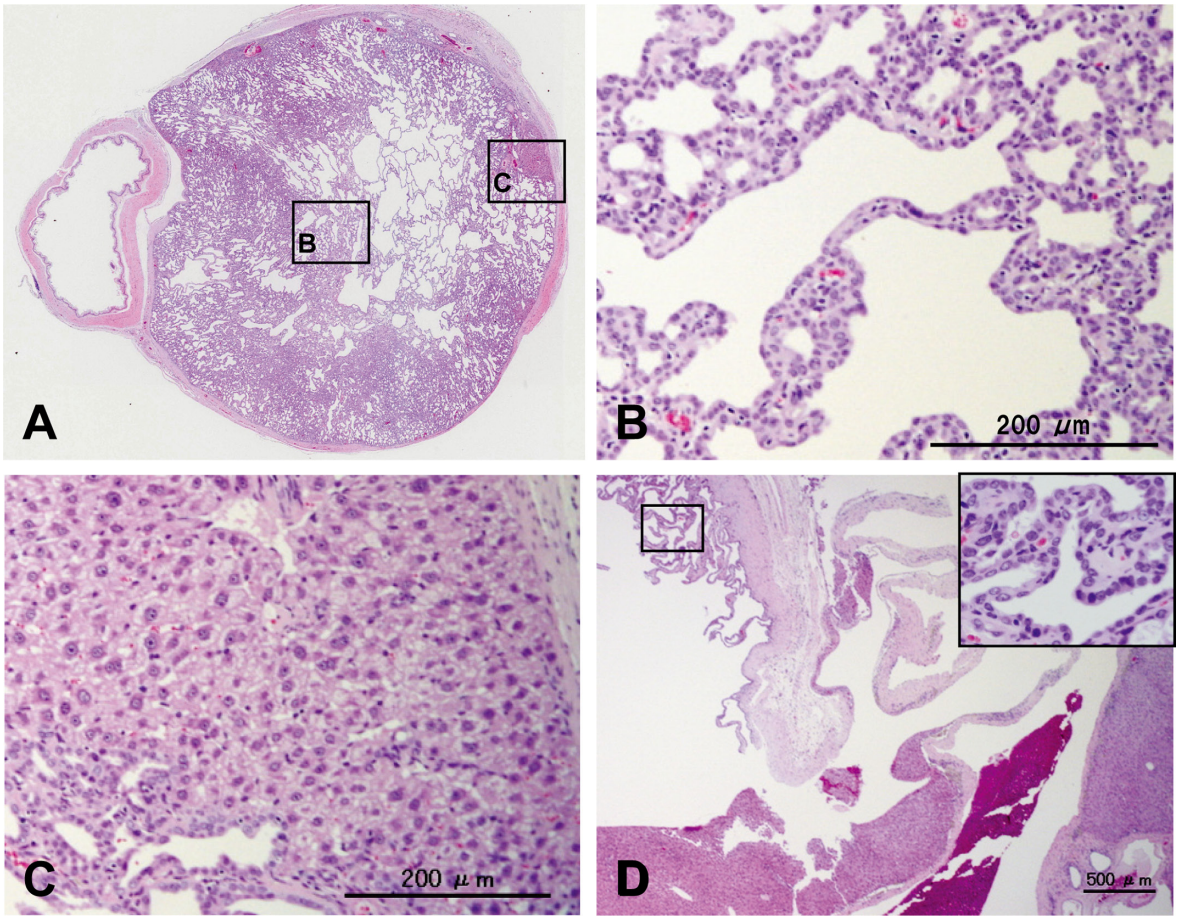

Fig. 2. Histological findings of the white mass. The mass was clearly circumscribed by connective tissue (A), and the neoplastic cells showed cystic tubular proliferation (B). Some islands of well-differentiated hepatocyte-like cells were observed in the mass (C). In the adhesion area between the liver and the diaphragm, the component cells were very similar to the tumor cells in the mass (D). HE stain.

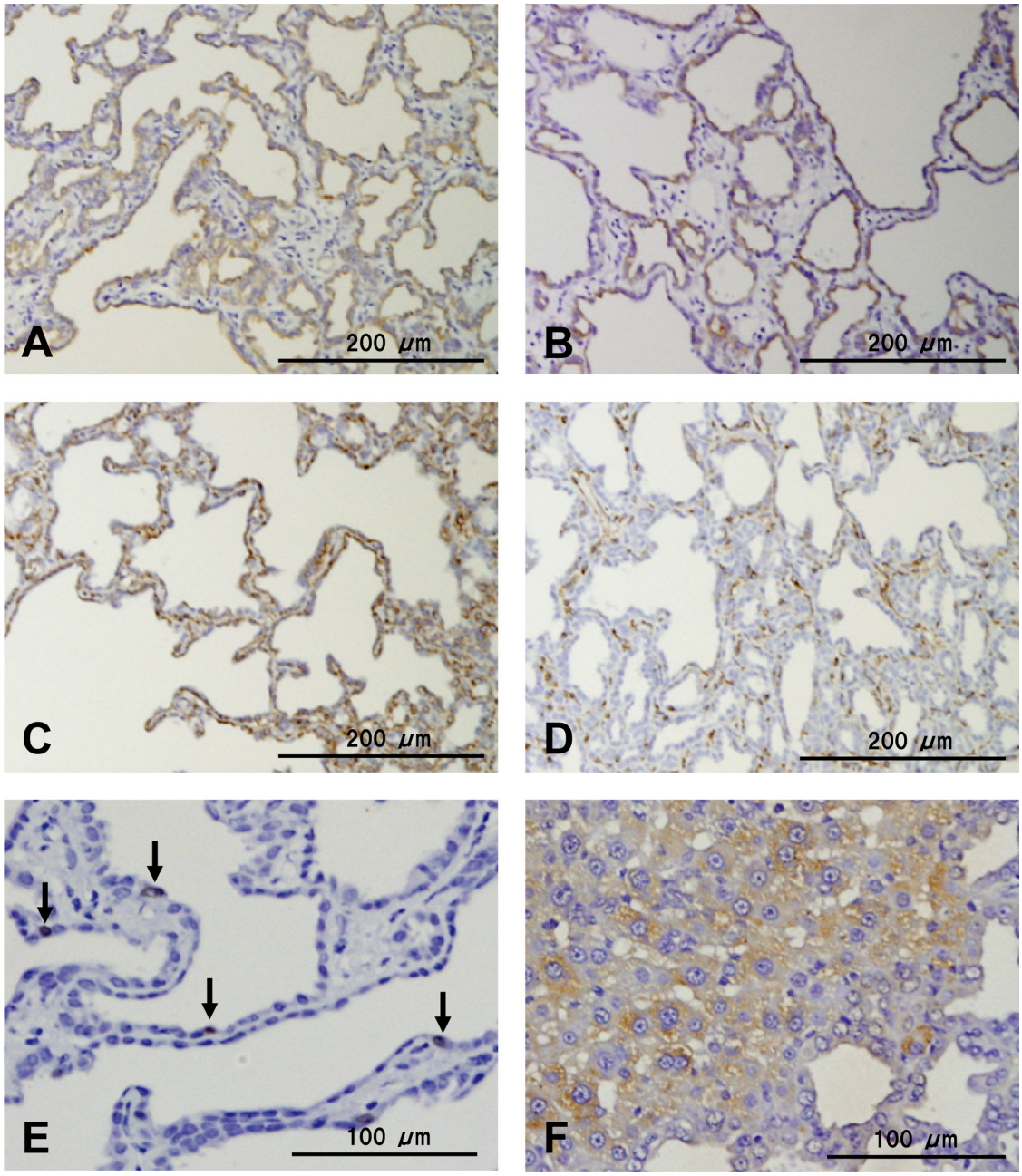

Fig. 3. Immunohistochemical findings of the white mass. The tumor cells were strongly positive for cytokeratin (A) and CK20 (B), partly positive for vimentin (C: positive area, D: negative area) and slightly positive for Ki-67 (E: Arrows indicate the positive cells.). The hepatocyte-like cells were positive for albumin (F).

tween the liver and the diaphragm, the component cells were very similar to the tumor cells in the mass (Fig. 2D), but a connection between the tumor and the liver was not detected. Any other significant histological findings were not observed in the esophagus, the diaphragm and the other areas of the liver. Immunohistochemically, the tumor cells were strongly positive for cytokeratin (Fig. 3A) and CK20 (Fig. 3B) and partly positive for vimentin (Fig. 3C, 3D) but were negative for albumin, mesothelin and Von Willbrand Factor. The percentage of Ki-67-positive tumor cells was 2.4% (Fig. 3E). The hepatocyte-like cells were positive for albumin (Fig. 3F) but were negative for cytokeratin and CK20. Based on these histological and immunohistochemical evidences, we diagnosed the white mass as a cystic cholangioma.

For the following reasons, the present case is considered to be appreciably rare. First, cystic cholangioma is a rare tumor of control rodents[1,2,4]. Second, the cholangioma in the present case was observed in the thoracic cavity despite the fact that cholangioma generally is considered to arise at or adjacent to the liver[1,2,4]. In the present case, though the histological features of the adhesive area between the

liver and the diaphragm were very similar to those of the tumor, an obvious connection between the tumor and the liver could not be confirmed. On the other hand, the histological features of the tumor and the result of anti-Ki-67 immunostaining indicated that the behavior of the tumor was clearly benign. So, we judged this tumor as a benign tumor and considered that the features in the adhesive area were not invasion or metastasis. Additionally, we suspected that the cause of the ectopic hepatic tissue in the thoracic cavity of the animal might have been a diaphragmatic hernia and that this tumor might have arisen from the ectopic hepatic tissue. Diaphragmatic hernia is generally caused by a birth defect or a traumatic accident. In the present case, the diaphragmatic hernia was considered to be a congenital lesion because no unexpected accidents in life and no gross findings indicating trauma were observed. In humans, some cases of tumorigenesis from ectopic hepatic tissue caused by diaphragmatic hernia have been reported, for example, hepatocellular adenoma[5], hemangioepithelioma[6] and hamartoma[7]. On the other hand, to our knowledge, no similar cases in rats have been reported previously.

As a differential diagnosis for our case, a hepatic hamartoma was considered. In the case report of a human case of hepatic hamartoma[7], the tissue consisted of bile duct-like structures, islands of well-differentiated hepatocytes and fibrous stroma. These components could all be seen together in the mass, and it was possible to find areas in which the hepatocytes could be seen in continuity with the bile duct structures. On the other hand, in our case, the majority of the mass was proliferative cuboidal or flattened epithelial cells, and the islands of hepatocyte-like cells were sporadically observed at margins of the mass. Furthermore, continuity between the hepatocyte-like cells and the tumor cells could not be observed at all. Therefore, we excluded the possibility of a hepatic hamartoma.

Immunohistochemically, the tumor cells in the present case coexpressed cytokeratin and vimentin. In general, vimentin is known to be a specific marker for mesenchymal cells. Some types of tumors such as carcinosarcoma, mesothelioma, mixed tumors of the salivary gland, müllerian tumor, teratoma and Wilms tumor have been known to coexpress cytokeratin and vimentin[8], but cholangioma has not been reported. Additionally, the malignancy of the tumor in the present case was considered not to be so high based on the results of the pathological examination. So, the reason for the positive expression of vimentin in the present case remains to be identified. On the other hand, Nakanuma *et al.* reported that vimentin was frequently positive in epithelial cells of proliferating bile ductules and interlobular bile ducts in various hepatobiliary diseases[9]. Furthermore, Milani *et al.* reported that vimentin expression was observed in epithelial cells of newly formed bile ductules in a rat model of secondary biliary fibrosis and also in normal rats[10]. These reports proposed that expression of vimentin might be related to proliferative or regenerative activity of bile duct epithelial cells.

In conclusion, the present case was diagnosed as a cystic cholangioma based on histological and immunohistochemical findings. To our knowledge, a cystic cholangioma in the thoracic cavity in a rat has never been reported, and the present case is considered to be appreciably rare.

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References

1. Narama I, Imaida K, Iwata H, Nakae D, Nishikawa A, and Harada T. A review of nomenclature and diagnostic criteria for proliferative lesions in the liver of rats by a working group of the Japanese society of toxicologic pathology. J Toxicol Pathol. **16**: 1–17. 2003. [CrossRef]
2. 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**: 5S–81S. 2010. [Medline] [CrossRef]
3. Goodman DG, Maronpot RR, Newberne PM, Popp JA, and Squire RA. Proliferative and selected other lesions in the liver of rats. G1–5. In: Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington DC. 1–15. 1994.
4. Okimoto K, Kouchi M, Kikawa E, Toyosawa K, Koujitani T, Tanaka K, Matsuoka N, Sakurai J, and Hino O. A novel "Nihon" rat model of a Mendelian dominantly inherited renal cell carcinoma. Jpn J Cancer Res. **91**: 1096–1099. 2000. [Medline] [CrossRef]
5. Shellito JG, and Bartlett WC. Diaphragmatic hernia; Traumatic, with herniation of tumor of liver. J Kans Med Soc. **59**: 51–53. 1958. [Medline]
6. Shah KD, Beck AR, Jhaveri MK, Keohane M, Weinberg B, and Gerber MA. Infantile hemangioendothelioma of heterotopic intrathoracic liver associated with diaphragmatic hernia. Hum Pathol. **18**: 754–756. 1987. [Medline] [CrossRef]
7. Deutsch AA, Brown KN, Freeman NV, and Stanley DA. A case of diaphragmatic hernia, absent pericardium, and hamartoma of liver. Br J Surg. **59**: 156–158. 1972. [Medline] [CrossRef]
8. McGuire LJ, Ng JP, and Lee JC. Coexpression of cytokeratin and vimentin. Appl Pathol. **7**: 73–84. 1989. [Medline]
9. Nakanuma Y, and Kono N. Expression of vimentin in proliferating and damaged bile ductules and interlobular bile ducts in nonneoplastic hepatobiliary diseases. Mod Pathol. **5**: 550–554. 1992. [Medline]
10. Milani S, Herbst H, Schuppan D, Niedobitek G, Kim KY, and Stein H. Vimentin expression of newly formed rat bile duct epithelial cells in secondary biliary fibrosis. Virchows Arch A Pathol Anat Histopathol. **415**: 237–242. 1989. [Medline] [CrossRef]