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Case Report

Dedifferentiated retroperitoneal liposarcoma spontaneously occurring in an aged SD rat

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Abstract: Liposarcoma is a rare neoplasm in rats and is characterized by the presence of lipoblasts containing multiple cytoplasmic vacuoles. We encountered a rare type of liposarcoma in a male SD (Crj:CD(SD)IGS) rat during a long-term study to gather background data. At necropsy at 105 weeks of age, there was a large amount of fatty tissue covering the mesentery, pancreas, and retroperitoneum; a white nodule in the right kidney; and paleness of the liver. Microscopically, the tumor had a well-differentiated component and dedifferentiated high-grade component. Immunohistochemical and electron microscopic examinations revealed that the pleomorphic tumor cells retained the characteristics of lipoblasts. Distant or disseminated metastasis was also confirmed in various organs. A liposarcoma with these histological features is extremely rare in rats, and this is the first report of a highly metastatic dedifferentiated type of liposarcoma originating from the abdominal fat tissue in a rat. (DOI: 10.1293/tox.2017-0055; J Toxicol Pathol 2018; 31: 141–146)

Key words: liposarcoma, lipoblasts, SD rat, spontaneous

In humans, liposarcoma is a rare soft tissue neoplasm accounting for approximately 20% of all soft tissue sarcomas[1]. They are categorized into four subtypes according to WHO criteria[2]. Most of them are well-differentiated liposarcoma/atypical lipomatous tumors, and in about 10% of cases, the tumor cells progress to a dedifferentiated phenotype[3–5]. In rats, spontaneous liposarcoma is also very rare (less than 0.1%) and occurs primarily in skin/subcutaneous tissues, and only a few cases have been reported in the abdominal cavity[6, 7]. Liposarcoma in rats is characterized by presence of malignant or primitive fat-forming cells termed lipoblasts. It also contains giant cells and spindle-shaped stellate cells in the myxoid stroma and dedifferentiated pleomorphic cells that can be highly mitotically active. A liposarcoma with distant metastasis has not been reported in rats. This paper describes a case of spontaneous dedifferentiated liposarcoma with metastasis to various organs in an aged Sprague Dawley (SD) rat.

The male SD (Crj:CD(SD)IGS) rat was a non-treated animal in a long-term study to gather background data. The 50 male and 50 female SD rats in the study were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan); housed in stainless steel cages in a room ventilated with filtered fresh air at a temperature of 20–26°C, a relative humidity of 40–60%, and under a 12/12-hr light/dark cycle; and allowed free access to tap water and to a widely used standard pelletized diet for experimental rats (MF, Oriental Yeast, Tokyo, Japan). The study was approved by the Committees for Animal Experiments of Fujifilm Corporation. Blood samples were collected from the jugular vein at 54, 62, 79, and 89 weeks of age. Prior to sacrifice for necropsy, blood samples were collected via the abdominal aorta under deep anesthesia with isoflurane. All serum samples were analyzed by a JCABM6050 system (JEOL Ltd., Tokyo, Japan). Collected tissues including tumor foci were fixed in 10% phosphate-buffered formalin, processed routinely for microscopic examination of paraffin-embedded sections, and stained with hematoxylin and eosin. The tissue sections were also stained immunohistochemically with rabbit polyclonal anti-S100 antibody (Dako North America, Inc., Carpinteria, CA, USA), mouse monoclonal anti-desmin antibody (Dako North America, Inc., Carpinteria, CA, USA),

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and anti-αSMA antibody (Abcam Inc, Cambridge, MA, USA), and visualized by staining with diaminobenzidine tetrahydrochloride as the chromogen and counterstaining with Mayer's hematoxylin. For electron microscopy, small pieces of formalin-fixed tumor tissue samples were fixed with 2.5% phosphate-buffered glutaraldehyde, postfixed in 1% osmium tetroxide solution (pH 7.4), and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (JEM-1400EX, JEOL Ltd., Tokyo, Japan).

In the antemortem phase, body weight started to decrease from 98 weeks of age (988.5 g), and it then continued to decrease until the end of study (875.0 g). At 105 weeks of age, the rat showed a decrease in locomotor activity, had abdominal distension, became moribund, and was euthanized. In blood biochemistry, γGT increased gradually from 62 weeks of age, and lipid-related parameters (T-CHO, TG, and PL) increased slightly from 89 weeks of age.

At necropsy, there was approximately 40 cc of bloody ascites and a large amount of fatty tissue covering the mesentery, pancreas, and retroperitoneum (Fig. 1). The stomach was markedly distended with many white spindle-shaped spots noted on the surface of abdominal peritoneum. There were many elastic and solid tumor nodules including one nodule along the limiting ridge between the stomach and duodenum (Fig. 1), a white nodule on the right kidney (7 mm in diameter), and numerous nodules on all lobules of the liver, which showed pale discoloration and had a rough surface. White and solid fatty tissues were observed to varying extents on the xiphoid process of the sternum, omentum surrounding the pancreas, muscles surrounding the reproductive organs, retroperitoneal muscle, and mesentery of the jejunum and ileum after formalin fixation.

Microscopically, the white fatty-like nodules in the abdominal cavity were composed of lipogenic cells and spindle cells (Fig. 2). The tumor nodules at the gastroduodenal junction were mainly occupied by proliferating spindle cells in a storiform pattern within a fibrous stroma. The stroma in the submucosa and serosa was composed of lipocytes, lipoblasts, or spindle cells of varying size and shape. The spindle cells with numerous lipid-like small cytoplasmic vacuoles tended to aggregate around lipocytes in the submucosa close to the lamina muscularis mucosae (Fig. 2). The mitotic index varied between fields, although there were some areas identified within tumor cells where more than 5 mitoses were present. In the gastric mucosa, the amount of necrosis varied, and tumor cells infiltrated all layers. In the liver, the spindle cells were characterized by chromatin-rich oval nuclei and eosinophilic cytoplasm and were mainly found in sinusoids. Their proliferation was accompanied by disintegration of the hepatic structure and cellular degeneration. In the lung, the metastatic foci were composed exclusively of spindle cells, with lipoblasts noted in the alveoli. In the kidney, the white fatty-like nodule contained lipogenic cells, spindle cells, and lipoblasts (Fig. 2). In short, the main cell type was lipogenic cells with some spindle cells in the fatty-like nodules of the abdominal cavity; almost exclusively

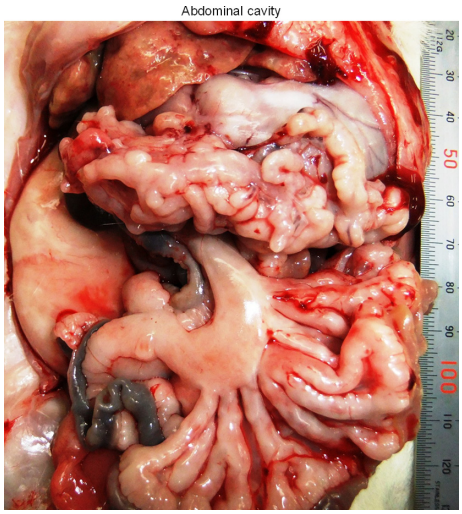

Fig. 1. Gross findings at necropsy. A picture image taken after laparotomy showing remarkably white fatty-like nodules in the abdominal cavity and nodules in various organs including liver, stomach, and pancreas. Blood ascites was removed before necropsy.

spindle cells in the stomach, liver, and lung, and lipogenic cells; and spindle cells with some lipoblasts in the tumor nodule of the kidney (Fig. 2).

Immunohistochemical staining for α-SMA, S100, and desmin was used to characterize the spindle cells of the tumor. The spindle cells were strongly positive for S100 protein (Fig. 3), while they were negative for desmin and α-SMA (data not shown). S100-positive spindle cells were diffusely distributed in the submucosal and muscle layers of the stomach (Fig. 3), concentrated in the sinusoids of the liver, and distributed from the periportal to centrilobular areas in the liver. Medium- to large-sized cytoplasmic vacuoles in lipocytes and lipoblasts and microvesicles in the cytoplasm of spindle cells in the submucosa, especially spindle cells around lipocytes, were positive for Oil Red O staining (Fig. 3).

Electron microscopic examination revealed moderately electron dense cytoplasmic lipid droplets of various sizes without a limiting membrane in the spindle cells, indicating their lipogenic origin, and the collagen fiber-like structure of the stroma surrounding the spindle cells (Fig. 4).

Regarding the metastasis in our study, blood vessels in the hepatic portal vein were histologically confirmed as the route of lipogenic cell invasion in the liver (Fig. 5) and other metastatic sites.

The differential diagnosis in the present case included dedifferentiated liposarcoma, pleomorphic undifferentiated sarcoma, fibrosarcoma, and high-grade myxofibrosarcoma. The transition from well-differentiated liposarcoma to high-grade pleomorphic liposarcoma in the present tumor fits well the definition of dedifferentiated liposarcoma. The sclerosing subtype reportedly shows scattered distinctive bizarre stromal cells associated with rare multivacuolated lipoblasts

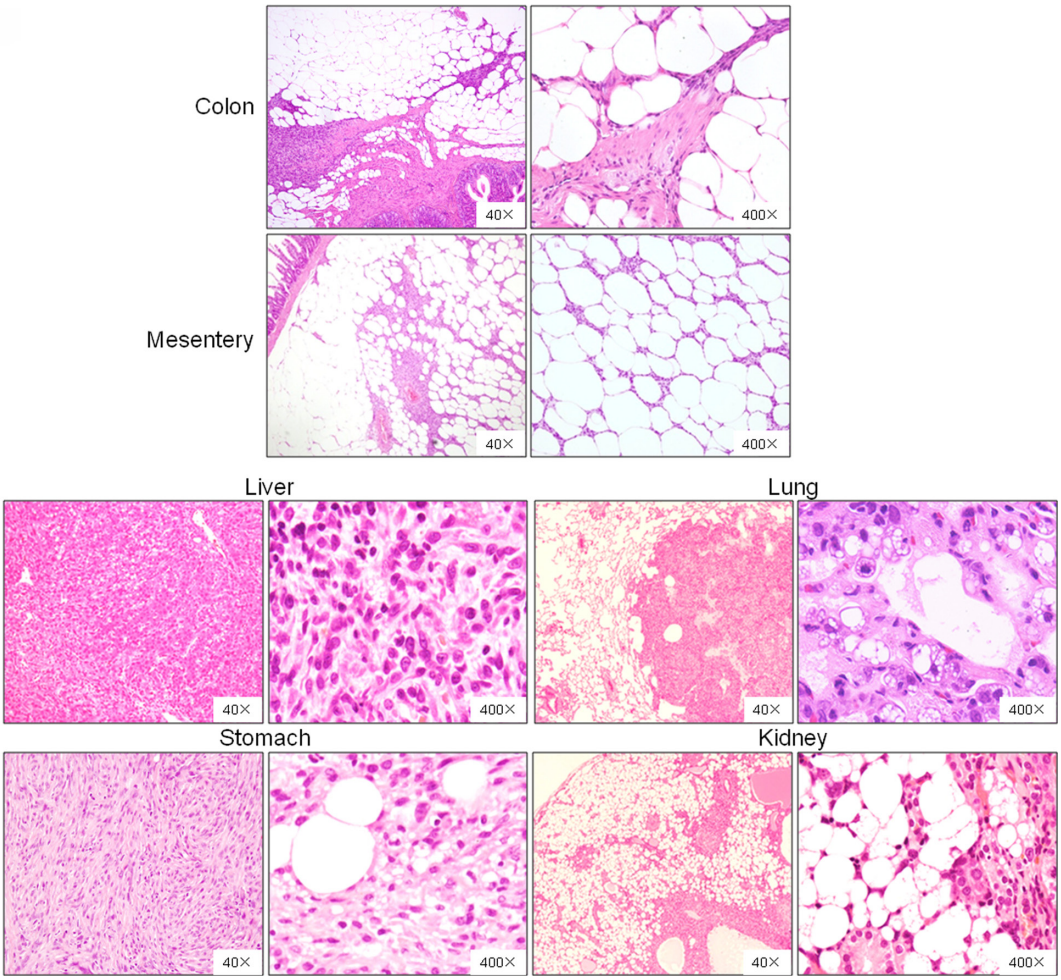

Fig. 2. Histology of tumor nodules in the colon, mesentery, liver, stomach, lung, and kidney. Images of H.E. stained tumor nodule specimens taken from the colon, mesentery, liver, stomach, lung, and kidney at low (40×, left panel) and high (400×, right panel) magnifications. An image of a tumor adherent to the colon and mesentery is representative of the white fatty-nodules in the abdominal cavity. By contrast, images of tumor nodules in the liver, lung, stomach, and kidney are representative of tumor metastasis. Tumor nodules adherent to the colon and mesentery are filled with atypical lipogenic cells and invasive spindle-shaped pleomorphic cells. Tumor foci in the liver, stomach, and lung, are predominantly spindle cells, while those in the kidney are scattered lipogenic spindle cells and lipoblast-like cells containing cytoplasmic lipid-like vacuoles.

set in a fibrillary collagenous background[8]. In our case, the transition between the dedifferentiated component and differentiated one was not clear, but its boundary was relatively distinct. The presence of coarse wavy collagen fibers, which are known as background components in liposarcomas, was ultrastructurally confirmed in part of the stroma of the present tumor. Oil Red O staining and electron microscopic examination revealed that the pleomorphic spindle cells had a high mitotic index and cytoplasmic lipid droplets of various sizes, and that they were of lipogenic origin. Metastasis was confirmed in the liver, lung, kidney, and stomach. Immunohistochemical findings of S100-positive liposarcoma with pleomorphic components as well as lipogenic cells further supported our diagnosis[9, 10] of dedifferentiated liposarcoma originating from the abdominal fat tissue with highly metastatic potential.

In the literature[11], liposarcoma in rats is histopathologically defined by the following criteria: fatty mass of variable appearance, fat-forming cells with a single large or multiple small cytoplasmic fat vacuoles, spindle cells, variable mitotic activity, and necrosis. The tumor in our case met these criteria as well as the criteria for diagnosing dedifferentiated liposarcoma in humans, including pleomorphic features (a mixture of differentiated and dedifferentiated types), presence of transitional cells (lipoblasts), frequent occurrence in abdominal retroperitoneal tissue, and high metastatic potential[12–15]. In summary, the tumor in our case had these pleomorphic features and metastasized to several organs. Therefore, the tumor in our case was finally diagnosed as dedifferentiated liposarcomas, which corresponded to the human tumor classification.

The WHO classification of soft tissue tumors describes four types of liposarcomas: (1) well differentiated/atypical lipomatous tumor, (2) dedifferentiated type, (3) myxoid/

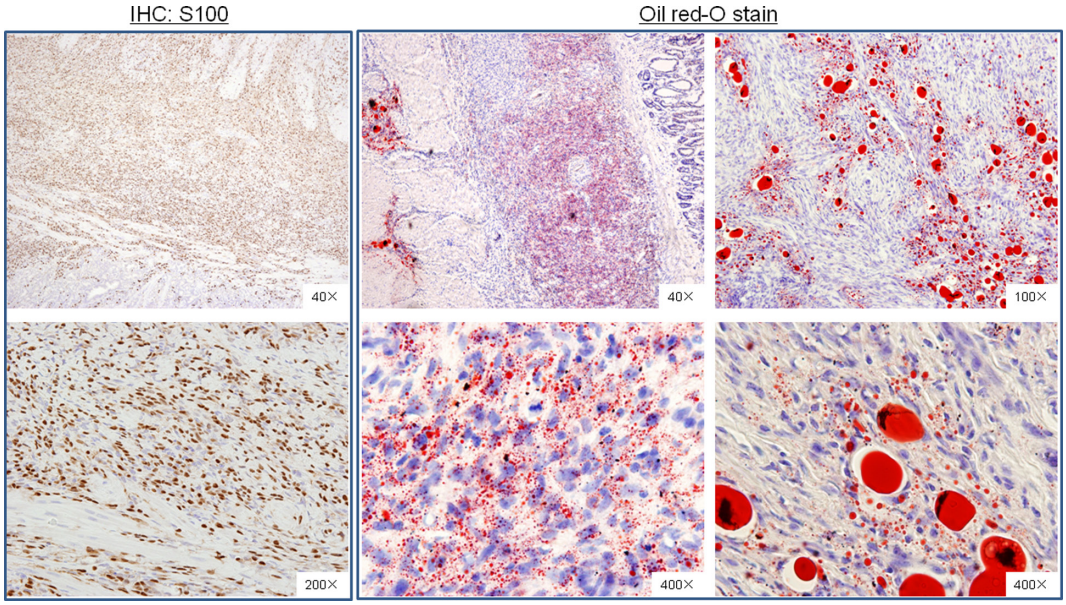

Fig. 3. S100 immunohistochemistry and Oil Red O staining of tumor nodules in the stomach. Spindle tumor cells were stained with anti-S100 antibody for rough diagnosis. S100 positive cells were found in the submucosal and subserous tissue in the stomach. Oil Red O staining showed the presence of lipid droplets (colored red) of various size in the tumor cells and foci located in the submucosa and serosa of the stomach. Magnification is indicated in the images.

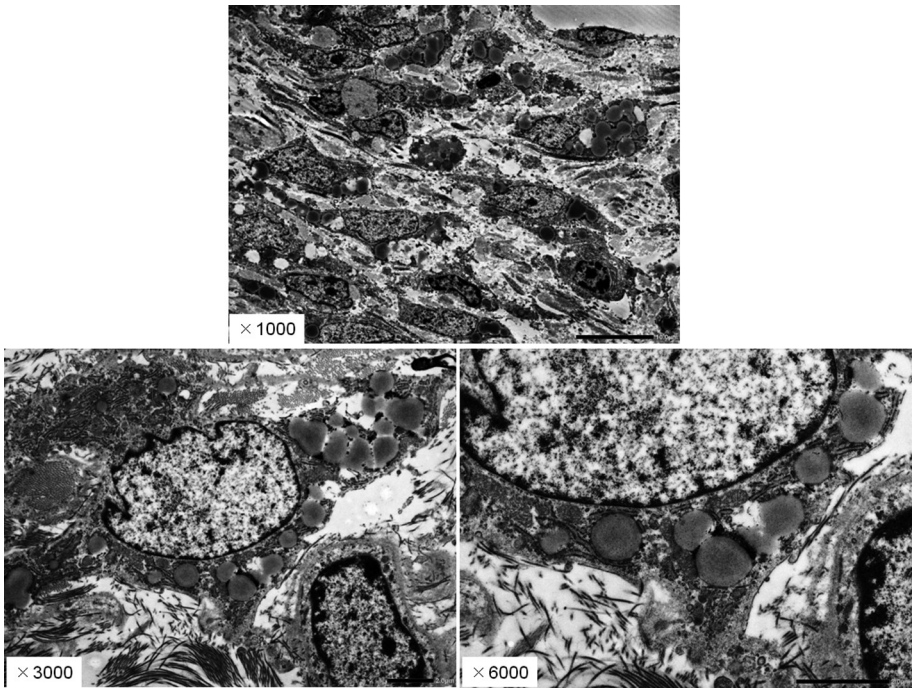

Fig. 4. Stomach spindle cell structure. Low magnification (1,000×) electron micrographs show moderately electron-dense lipid droplets of various size in the cytoplasm of almost all spindle cells, and collagen fibers localized in the stromal area between cell junctions.

round cell type, and (4) pleomorphic type[2, 3]. Well-differentiated liposarcomas masquerade as mature adipose tissue; however, they typically contain fibrous septa with scattered atypical cells[8, 16]. Dedifferentiated liposarcoma behaves in a more aggressive manner with a greater propensity for local recurrence and the capacity to metastasize. It is usually derived from high-grade pleomorphic areas adjoining a differentiated liposarcoma[17]. In our case, dedifferentiated pleomorphic components were prominent in the gastrointestinal tract, liver, and lung.

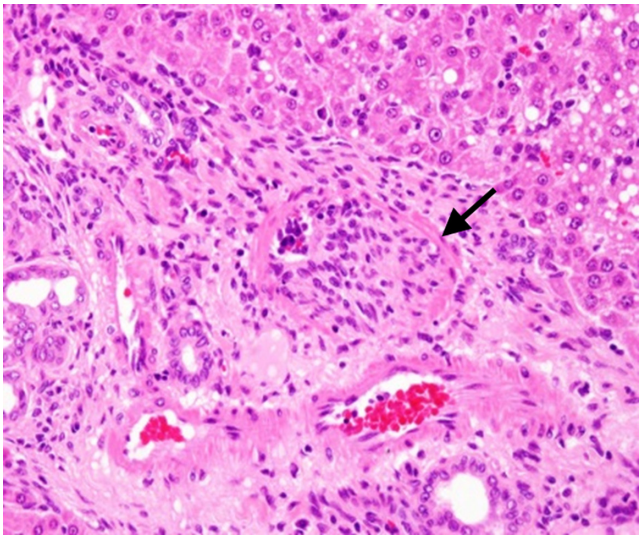

Fig. 5. Metastasis of lipogenic tumor cells via the blood circulatory system. Representative images show lipogenic tumor cells exiting the tumor via the blood system in the portal region of the liver. Arrows indicate the blood vessels containing the lipogenic tumor cells. Magnification is 400×.

Distant metastases of retroperitoneal dedifferentiated liposarcoma in liver and lung have been previously reported[18-20] and shown histologically to be dedifferentiated and without a well-differentiated component, which corresponds well to the metastases in our case.

Additionally, liposarcoma is one of the most common primary retroperitoneal neoplasms, and the perinephric region is a frequent location for them[21]. On macroscopic examination, the mass encircling the kidney is well encapsulated and is rubbery, greasy, and yellowish white when cut, giving it a lipoma-like appearance[22]. Histologically, the tumor is characterized by proliferating adipocytes of varied size and shape, occasional multivacuolated lipoblasts, and spindle-shaped cells with fat cells interspersed amongst spindle-shaped cells[22].

Therefore, based on the abovementioned reports that identified the origin of liposarcoma to be predominantly the retroperitoneum, we had assumed that this would also apply to this case, but we could not definitely identify the location of origin. Reports in the literature about the origin of liposarcoma also support the retroperitoneum as the origin[12, 23, 24].

Accordingly, it is proposed that liposarcoma originates from a site in the abdominal cavity and that cells from this site disseminate to many organs through the blood system. Evidence supporting this proposal includes the occurrence of nodules of lipogenic tumor cells in the abdominal cavity, transition from differentiated lipogenic cells to dedifferentiated lipogenic cells in the tumor foci, and metastatic spread via the blood system to various organs. However, the primary tumor in our specimen could not be identified with certainty due to progression of the tumor.

In conclusion, we described a dedifferentiated liposarcoma composed of well-differentiated and dedifferentiated areas. The peritoneal dissemination of this tumor was shown by the presence of metastasis in a wide range of organs. These histological features are extremely rare and have not yet been reported in rats. This is the first report of a highly metastatic dedifferentiated type of liposarcoma in a rat.

Disclosure of Potential Conflicts of Interests: There are no known conflicts of interest associated with this study.

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