Case Report

Spontaneous infarcted adenoma of the mammary gland in a Wistar Hannover GALAS rat

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Abstract: Spontaneous massive infarction of mammary gland tumors has been reported to occur infrequently in humans. A subcutaneous mass (18 × 17 × 10 mm) was observed in the right axilla extending to the chest region of a 110-week-old female Wistar Hannover GALAS rat. Histopathologically, a well-circumscribed mass with lobular structures was present in the subcutis. Most of the mass was occupied by extensive coagulative necrosis of neoplastic cells with relatively uniform acinar and ductal structures. Although each necrotic acinar structure was separated by reticular fibers, periacinar stromal collagen fibers were not abundant. Considering the site of occurrence and histological features, the necrotic tissue was diagnosed as adenoma of the mammary gland. The necrotic region lacked hemorrhage and obvious inflammatory cell infiltration, indicating the necrosis was caused by infarction. Although multiple necrosis and focal infarction are occasionally observed in large-sized tumors in rodents, especially in adenocarcinomas, the present case was characteristic, with the massive infarction involving most parts of the tumor despite the relatively small size and low atypia of neoplastic cells. This is a rare case of spontaneous infarcted adenoma of the mammary gland in rats histologically resembling human cases. (DOI: 10.1293/tox.2016-0042; J Toxicol Pathol 2017; 30: 57–62)

Key words: infarcted adenoma, mammary gland, spontaneous lesion, Wistar Hannover GALAS rat

Mammary gland tumors frequently occur in humans, companion animals, and experimental rodents. Although the diagnostic criteria differ among species, they share morphological similarities. Infrequently, massive infarction involving most parts of a tumor has been reported in human mammary gland tumors, particularly in fibroadenomas[1]. Most infarctions have occurred secondary to fine-needle aspiration or were associated with pregnancy or lactation[2, 3]. Spontaneous infarcted mammary gland tumor in the absence of known associated factors is uncommon[1, 4]. To the best of our knowledge, this is the first case report demonstrating an infarcted adenoma of the mammary gland in experimental rodents that histologically resembles those observed in humans.

The animal was a female Wistar Hannover GALAS rat (CLEA Japan Inc., Tokyo, Japan) allocated to a low-dose group in a 104-week feeding carcinogenicity study. The rat was fed a powder diet and tap water ad libitum and housed in a plastic cage on sterilized softwood chip bedding in a room with a barrier system maintained at 24 ± 1°C and 55 ± 5% humidity on a 12-h light/dark cycle. The experimental protocol was approved by the Animal Care and Utilization Committee of the National Institute of Health Sciences, Japan, and the animals were cared for in accordance with institutional guidelines as well as the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006). In the carcinogenicity study, no effects of test chemical treatment on the incidence of mammary gland tumor were observed in any group. Although mammary gland tumors, including fibroadenoma, adenoma, and adenocarcinoma, were detected sporadically in other animals, similar infarcted lesions were not observed among the tumors. Thus, the present case was considered to be a spontaneous lesion and not related to the test chemical treatment.

A subcutaneous mass of about 20 mm in diameter was detected on palpation in the right axilla extending to the chest of the female rat during clinical observation at 103 weeks of treatment. The female rat survived until the scheduled sacrifice (110 weeks of age) without any other clinical signs. At necropsy, the subcutaneous mass was soft, 18 × 17 × 10 mm in size, and well demarcated from surrounding tissues. The cut surface was white. Other macroscopic findings, including distention of the uterus, an abdominal cyst attached to the pancreas and liver containing calculi, unilateral enlargement of the mandibular lymph node, and

multiple white foci in the lungs, were not considered to be related to the subcutaneous mass.

The subcutaneous mass was fixed in 10% neutral-buffered formalin, and slices were routinely embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE). Masson's trichrome staining, silver impregnation, and immunohistochemistry were also performed. For immunohistochemistry, sections were immersed in 3% H_2O_2/methanol for inactivation of endogenous peroxidase activity. The following primary antibodies were used: mouse anti-ED1 (monoclonal, KP1, diluted 1:100; AbD Serotec, Oxford, UK), mouse anti-α-smooth muscle actin (SMA) (monoclonal, 1A4, diluted 1:50; Dako, Glostrup, Denmark), and rabbit anti-von Willebrand factor (vWF) (polyclonal, diluted 1:200; Dako). Antigen retrieval for ED1 and α-SMA was performed in 10 mM citrate buffer (pH 6.0) using an autoclave for 15 min at 121°C. Proteinase K (ready to use; Dako) digestion at room temperature for 15 min was used for antigen retrieval for vWF. After blocking of nonspecific reactions with 10% normal goat serum, the sections were incubated with each primary antibody overnight at 4°C. Visualization was performed using a Histofine Simple Stain Rat MAX PO kit (Nichirei Corporation, Tokyo, Japan) and 3,3′-diaminobenzidine. All sections were counterstained with hematoxylin. Oil red O staining was also performed using a frozen section of the formalin-fixed mass sample.

On histopathological examination, a well-circumscribed mass with a lobular structure was observed in the subcutis. The mass consisted of four main components: a diffuse necrotic area that occupied most parts of the mass, a relatively small focus of the residual tumor, a granulomatous lesion surrounding the outer edge of the necrotic area, and immature granulation tissues (Fig. 1). The main part of the mass was occupied by extensive coagulative necrosis characterized by relatively uniform acinar and ductal neoplastic cells with pyknotic nuclei or only ghostly outlines containing vacuoles of various sizes (Fig. 2A, B). Oil red O staining revealed that these vacuoles were lipid droplets (Fig. 2B). Necrotic tumor cells were divided by reticular fibers into the acinar and ductal structures as shown by silver impregnation (Fig. 2C). No abundant periacinar stromal collagen fibers, obvious inflammatory cell infiltration, or hemorrhage in the necrotic region were observed (Fig. 2A–2D). A small focal proliferative region of neoplastic cells was present at the periphery of the extensive necrotic area (Fig. 3A). Epithelial neoplastic cells with cytoplasmic vacuoles were arranged
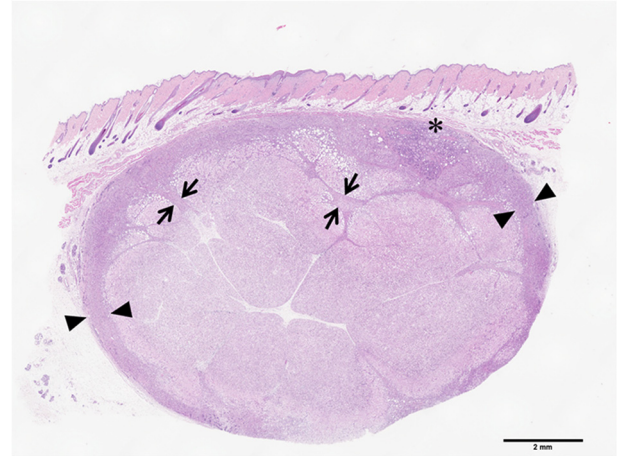

Fig. 1. Whole-mount histopathology of the subcutaneous mass created by image stitching function using an All-in-One Fluorescence Microscope BZ-X710 (KEYENCE Corporation, Osaka, Japan). A well-circumscribed mass with a lobular architecture was present in the subcutis. Most parts of the mass were occupied by massive coagulative necrosis. Asterisk: a focal proliferative region of residual neoplastic cells at the periphery of the necrotic lesion. Arrowheads: a granulomatous lesion surrounding the outer edge of the necrotic area. Arrows: granulation tissues proliferating in the necrotic region.

in monolayered acinar or ductal structures, frequently containing intraluminal secretion (Fig. 3B). The neoplastic cells showed mild nuclear atypia and several mitotic figures, and the alveolar structure was slightly distorted. Periacinar proliferation of fibrous tissue was rarely detected (Fig. 3C). The necrotic tissue was almost entirely surrounded by thick granulomatous lesions (Fig. 4A). The granulomatous lesions were composed of an aggregation of epithelioid cells and multinucleated giant cells with vacuolated cytoplasm (Fig. 4B). The vacuoles were revealed to be lipid droplets similar to those observed in necrotic neoplastic cells (Fig. 4B). The aggregated cells were immunohistochemically positive for ED1 (Fig. 4C), indicating macrophage origin. A number of capillary blood vessels positive for vWF were observed in the granuloma, particularly in the margin of the necrotic region (Fig. 4D). Granulation tissues were present in the necrotic area, primarily in the interlobular region (Fig. 5A, B). A number of vWF-positive neovascular vessels were also observed in the granulation tissues (Fig. 5C). Proliferating

Fig. 2. Necrotic region in the central part of the subcutaneous mass. (A) Relatively uniform acinar and/or ductal structures showed extensive coagulative necrosis without obvious inflammatory cell infiltration or hemorrhage. (B) Higher magnification. Necrotic neoplastic cells had cytoplasmic vacuoles of various sizes. Oil red O staining revealed the vacuoles to be lipid droplets (insert). (C) Silver impregnation showed a reticular framework in the necrotic regions. (D) Periacinar proliferation of stromal connective tissue is not abundant, as observed with Masson's trichrome staining.

Fig. 3. Proliferative lesion of residual neoplastic cells at the periphery of the necrotic area. (A) Neoplastic epithelial cells formed acinar or ductal structures with intraluminal secretions. (B) Higher magnification. Monolayered alveolar structures were lined by neoplastic epithelial cells with intracytoplasmic vacuoles accompanied by scant stromal connective tissue. The neoplastic cells showed mild cellular atypia and several mitotic figures, and the ductal structure was slightly distorted. (C) Masson's trichrome staining demonstrated a lack of abundant periacinar stromal proliferation.
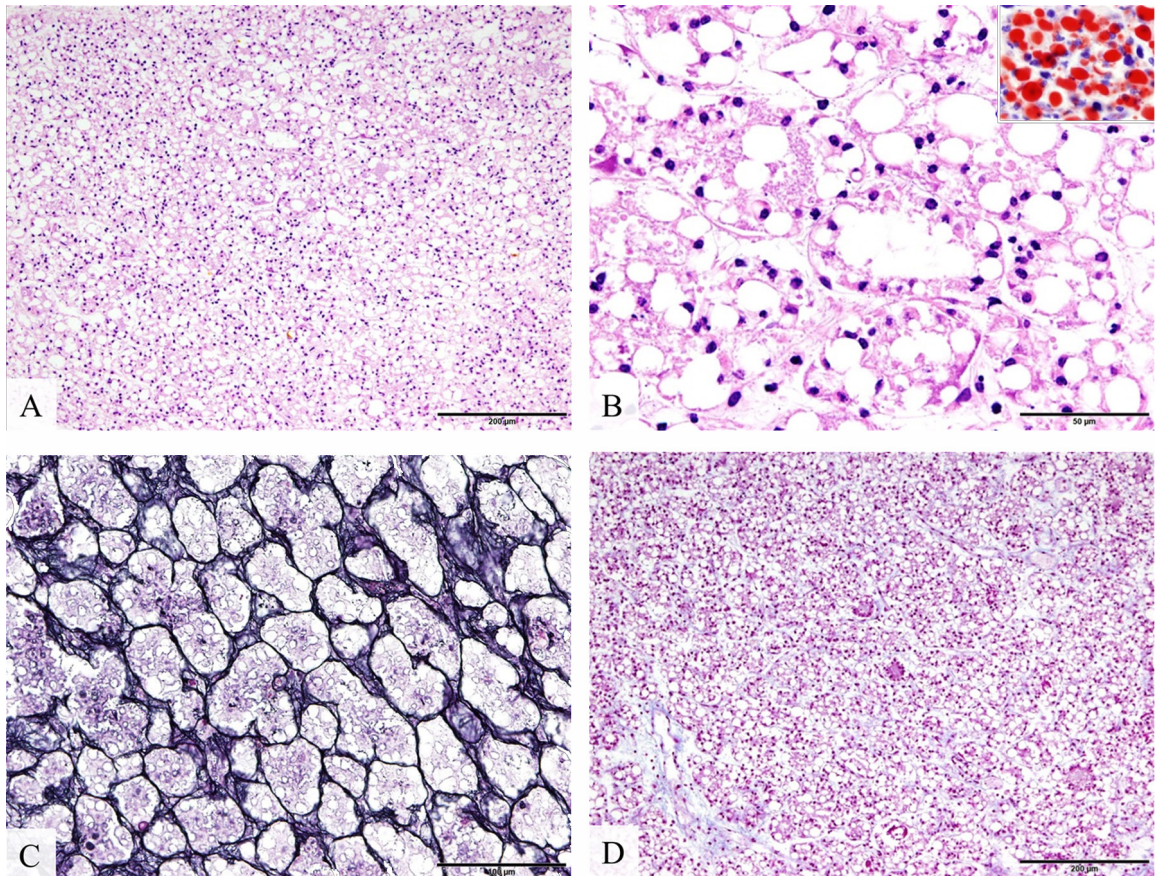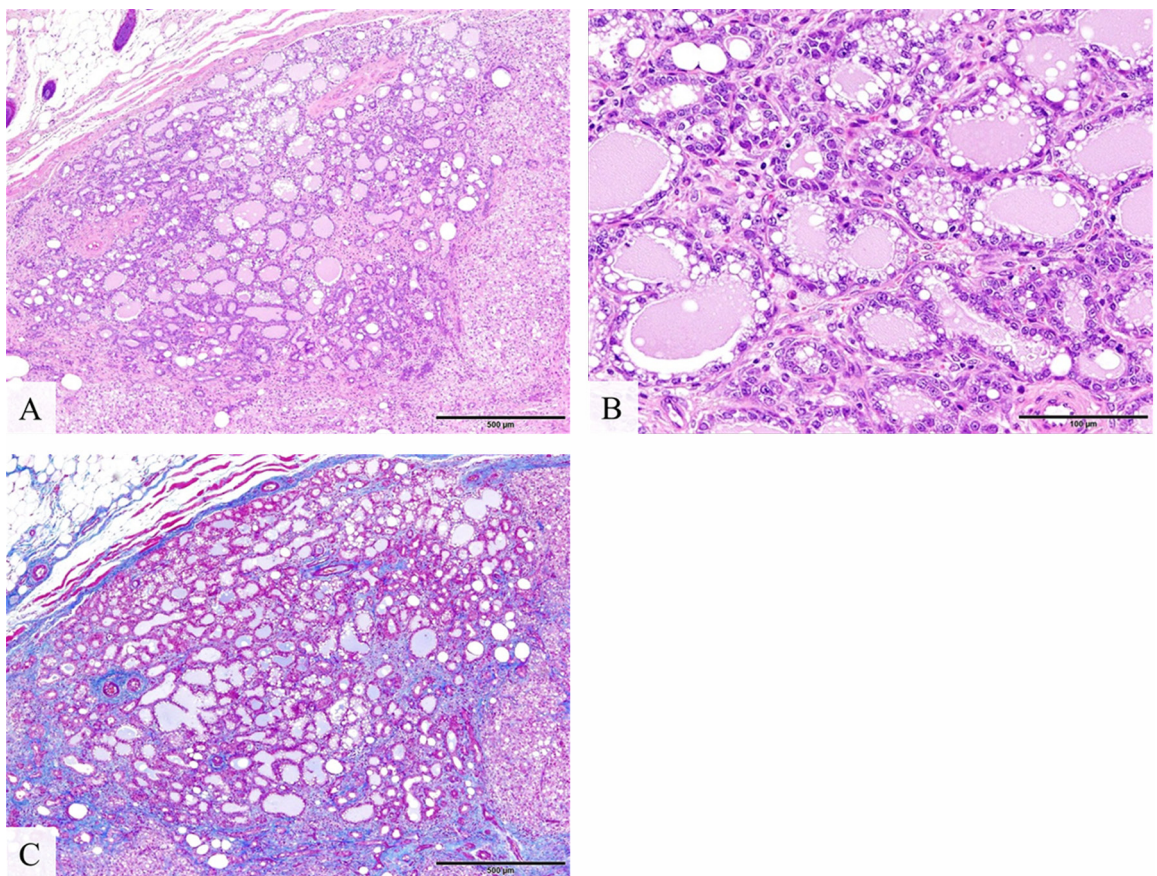
Fig. 2.

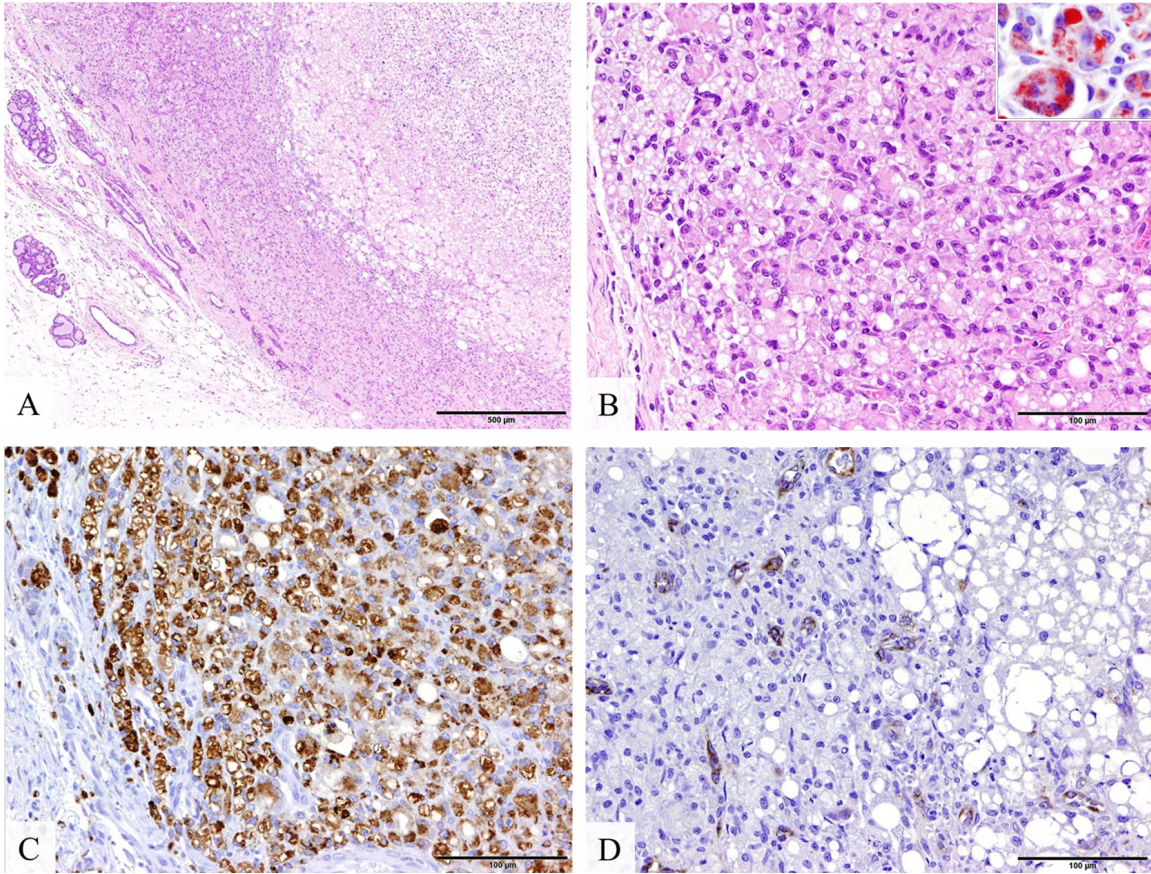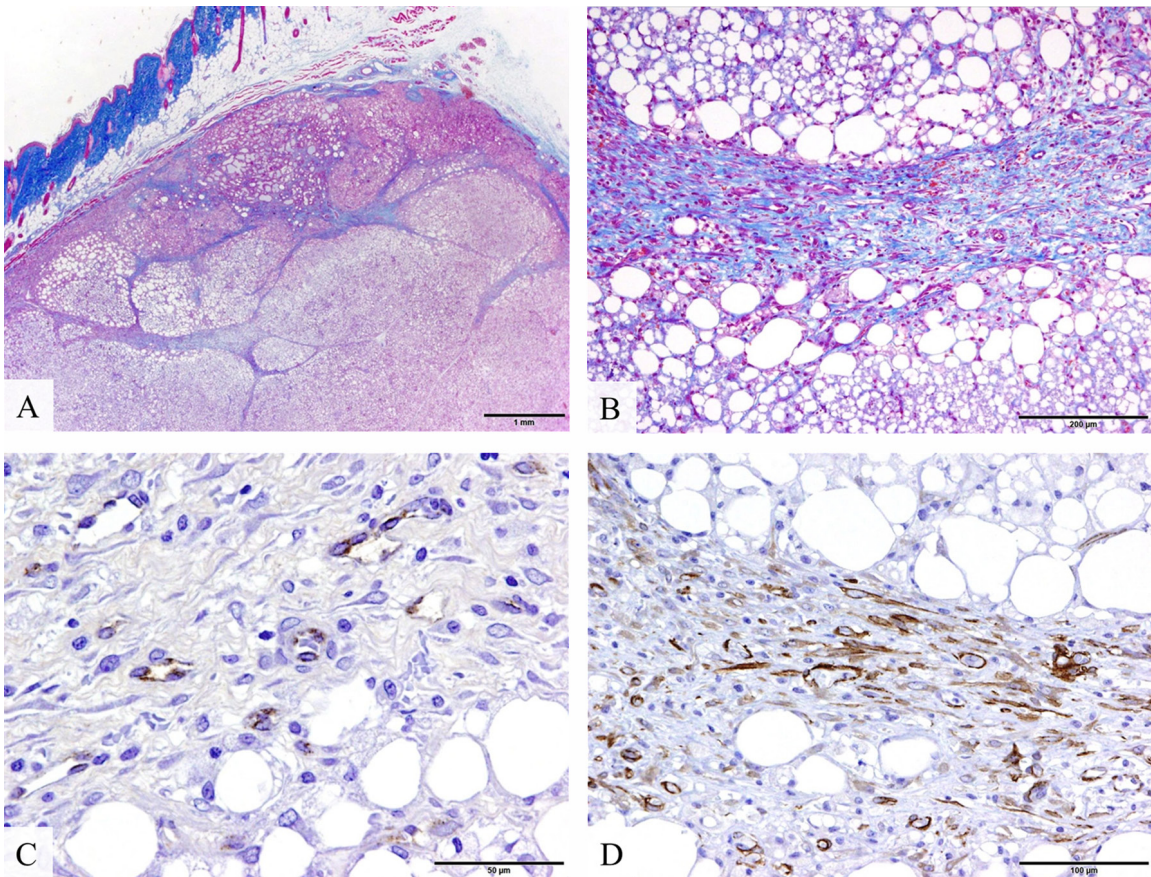**Fig. 3.**

Fig. 4.

Fig. 5.

α-SMA-positive myofibroblasts and vascular smooth muscle cells were also seen (Fig. 5D). No infarcted lesions, vasculitis, or thrombus formation in any other organs, including the heart and kidney, common target sites of systemic thrombosis, were observed.

In human pathological studies, massive infarction is infrequently observed in breast tumors, including fibroadenomas, phyllodes tumors, intraductal papillomas, lactating adenomas, and ductal adenomas[1, 5–8]. Most case reports have demonstrated infarction of fibroadenomas, the most common benign breast tumors in women[9]. Histopathologically, the reported cases are characterized by extensive coagulative necrosis of tumor tissue with adjacent foamy histiocytes, fibrosis, and residual epithelium, similar to the present case[10].

In the present case, the subcutaneous mass primarily consisted of coagulative necrotic tissue without obvious inflammatory cell infiltration or hemorrhage, indicating that the extensive necrosis could have been caused by acute and complete interruption of blood flow, i.e., infarction. Although detailed morphological features of the necrotic cells such as cellular atypia or mitotic figures could not be analyzed, the necrotic cells were constructed of relatively uniform acinar and ductal structures with scant proliferation of stromal tissues. Given the site of occurrence and histopathological features, the necrotic tissue is considered to be derived from an adenoma of the mammary gland, specifically, the alveolar/tubular subtype[11]. Small focal neoplastic tissue at the periphery of the mass also showed morphologic features of mammary gland adenoma, including cellular and structural atypia with scant connective tissue. The adenomatous focus was most likely a part of the main tumor of the infarcted adenoma and possibly avoided necrosis caused by infarction due to an alternative blood supply. Granulomatous lesions surrounding the mass could have developed to eliminate the necrotic neoplastic cells. Neovascularity in the granuloma and formation of immature granulation tissues suggest that the necrotic tissue was in the early stages of organization. Based on the histopathological findings, the mass was diagnosed as an infarcted adenoma of the mammary gland.

Skenderi *et al.* showed that massive infarction occurs in about 0.3% of human fibroadenomas[1]. Most cases of infarction of fibroadenomas have been reported to be secondary to fine-needle aspiration[2]. Fine-needle aspiration also induces infarction in other organs, such as the salivary gland, thyroid gland, and lymph node[12–14]. Mechanisms of infarction induced by fine-needle aspiration are considered to be associated with interruption of the microvascular supply by direct vascular damage or traumatic thrombosis[13]. In contrast, spontaneous infarction may occur in fibroadenomas or physiologic hyperplasia during pregnancy or lactation[3, 15–17]. Insufficient vascular formation of a rapidly growing tumor or hyperplastic tissue in these conditions is the widely accepted hypothesis for the etiology of spontaneous infarction in the mammary gland[18]. Spontaneous infarction of neoplasms has also been reported in the pituitary gland, although detailed mechanisms remain uncertain[19].

Multiple necrosis and focal infarction are occasionally observed in large-sized mammary gland tumors in rodents, especially in adenocarcinomas[11]. The present case was characteristic, with the massive infarction involving most parts of the tumor despite the relatively small size and low atypia of neoplastic cells. To the best of our knowledge, no reports demonstrating spontaneous massive infarction of mammary gland tumors in companion animals or experimental rodents have been published. Most human cases of infarcted mammary gland tumor involve fibroadenomas. In addition, spontaneous infarction of fibroadenomas without a clear etiology, such as fine-needle aspiration or pregnancy/lactation, is extremely uncommon. The present report demonstrated an infarcted adenoma of the mammary gland in a nonpregnant female rat histopathologically resembling human cases. Possible causative factors such as vasculitis or thrombus formation were not observed in the present case. The rat did not have a pituitary tumor, which could have possibly induced proliferation of mammary gland tissue via hyperprolactinemia. Mammary gland tumors that occurred in other animals in this carcinogenicity study were first detected as subcutaneous masses of about 20 mm in diameter on palpation, similar to the present case. Thus, there are little evidence implying that the infarction of the present case was associated with rapid growth of neoplastic tissue. Although the detailed pathogenesis remains uncertain, this report is of interest due to the pathological characteristics reported herein.

The present case was considered to be at a relatively early stage after infarction because of the remaining acinar and ductal structure and lack of obvious hemorrhage and inflammatory cell infiltration in the necrotic area, whereas elimination and repair processes such as aggregation of macrophages and formation of granulation tissue were observed

Fig. 4. Granulomatous lesions surrounding the necrotic area. (A) The necrotic region was almost entirely surrounded by granulomatous tissues. Normal mammary gland tissues existed adjacent to the mass. (B) Higher magnification. The granulomatous lesion was composed of an aggregation of macrophages and multinucleated giant cells with cytoplasmic vacuoles. The vacuoles were identified as lipid droplets by Oil red O staining (insert). (C) The macrophages accumulated around the mass were positive for ED1 on immunohistochemistry. (D) A number of vWF-positive small blood vessels were formed in the granuloma, particularly in the margin of the necrotic region.

Fig. 5. Granulation tissues in the necrotic region. (A) Masson's trichrome staining showed that fibrous tissues had developed into the necrotic area, mainly in the interlobular region. (B) Higher magnification. Collagenous connective tissues were formed among the necrotic lobules. (C) A number of vWF-positive small blood vessels were observed in the granulation tissues. (D) Myofibroblasts and vascular smooth muscles were immunohistochemically positive for α-SMA.

mainly at the periphery of the infarcted lesion. If massive infarction of mammary gland tumors occurs in an earlier period of a toxicological study, unexpected regression of a subcutaneous mass can be detected in clinical observation. In fact, regression or resolution of fibroadenomas possibly due to infarction has been reported in human pathological studies[20]. In addition, it is difficult to specify the origin on an infarcted adenoma completely replaced by granulation tissue or a scar. The information in this case report could be useful for interpretation and discussion of such possible cases.

Disclosure of Potential Conflicts of Interest: Mizuki Sone is an employee of Kao Corporation, Tokyo, Japan. The authors declare that there are no other conflicts of interest.

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