

Case report

Spontaneous Mammary Adenocarcinoma in a Twelve-week-old Female Sprague-Dawley Rat

Kazuya Kuzutani^{1*}, Toshiyuki Shibunushi¹, Yumi Kangawa¹, and Tohru Kihara¹

¹ Kaken Pharmaceutical Co., Ltd., Pharmacokinetics and Safety Research Department, 301 Gensuke, Fujieda-Shi, Shizuoka 426-8646, Japan

Abstract: Spontaneous mammary adenocarcinoma was observed in a 12-week-old female SD rat. A movable mass in the right cervical region was found at 11 weeks of age, and the rat was sacrificed the following week. The mass was located in the vicinity of the right salivary gland and measured 38 mm × 26 mm × 16 mm in gross size. It was a firm whitish mass, with a cut surface that was also whitish in appearance. Histopathologically, neoplastic cells formed glandular structures that contained secreted eosinophilic material. Ultrastructurally, similar secreted material and lipid droplets were in the cytoplasm of the neoplastic cells. Immunohistochemically, the neoplastic cells were positive for cytokeratin 8, cytokeratin 18 and estrogen receptor α . Based on these findings, the tumor was diagnosed as a mammary gland adenocarcinoma, and we therefore conclude that this tumor type can occur spontaneously in female SD rats as young as 12 weeks of age. (DOI: 10.1293/tox.25.221; *J Toxicol Pathol* 2012; 25: 221–224)

Key words: mammary gland, adenocarcinoma, young rat

Mammary tumors are recognized as one of the most common tumors occurring spontaneously in aging female rats^{1–4}. In general, mammary tumors occur with increasing frequency as animals age^{5, 6}, however, they are rare before one year of age⁶. There are only a few reports of mammary tumors in young rats, particularly under 20 weeks of age^{7, 8}. This paper describes mammary adenocarcinoma in a 12-week-old female SD rat.

Ten-week-old male and female Sprague-Dawley rats (CrI:CD(SD)) were purchased from Charles River Laboratories Japan Inc. (Shiga, Japan) for use in a reproductive toxicity study. The rats were housed in a wire mesh cage in a room used to keep animals and under had controlled conditions (23 ± 3 degrees C and a relative humidity of 50 ± 20%) with free access to a commercial chow (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and fresh water. Animals were handled according to the animal care guidelines of Kaken Pharmaceutical Co., Ltd. (Shizuoka, Japan). In the acclimation period (7 days), there were no abnormal findings in the rat, but we discovered a movable mass in the right cervical region 10 days after receipt, and then sacrificed the animal the following week. The mass was located in the vicinity of the right submandibular and sublingual gland.

It was whitish and firm, measuring 38 mm × 26 mm × 16 mm in gross size, and the cut surface of the mass was also whitish in appearance. The submandibular and sublingual glands were normal, and no abnormalities were found in other organs. For routine histopathologic examination, the mass was fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at a thickness of 4 μ m, and stained with hematoxylin and eosin. Additional sections of the mass were stained with periodic acid-Schiff (PAS) stain and analyzed immunohistochemically by staining with primary antibodies against cytokeratin 8 (CK8) (undiluted; PROGEN, Biotechnik GmbH, Heidelberg, Germany), cytokeratin 18 (CK18) (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and estrogen receptor α (ER- α) (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Antigen retrieval was performed in citrate buffer (PH 6.0) using an autoclave, and then samples were incubated with the antibodies at room temperature for 5 h (CK8), 1.5 h (CK18) or 20 min (ER- α), followed by incubation in secondary antibody and streptavidin at room temperature for 10 min using LSAB-2 System-HRP (DAKO). Visualization was performed with diaminobenzidine. For transmission electron microscopy, small blocks were prefixed in buffered 2.5% glutaraldehyde, postfixed in buffered 1% osmium, routinely processed into epoxy resin-embedded ultrathin sections and stained with uranyl acetate and lead citrate.

Histopathological examination showed that the tumor was densely cellular and surrounded by a thick capsule with bands of collagen extending into the parenchyma, and a normal mammary gland and parotid gland were present in the vicinity of the tumor (Fig. 1A). The tumor consisted

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*Corresponding author: K. Kuzutani (e-mail: kuzutani_kazuya@kaken.co.jp)

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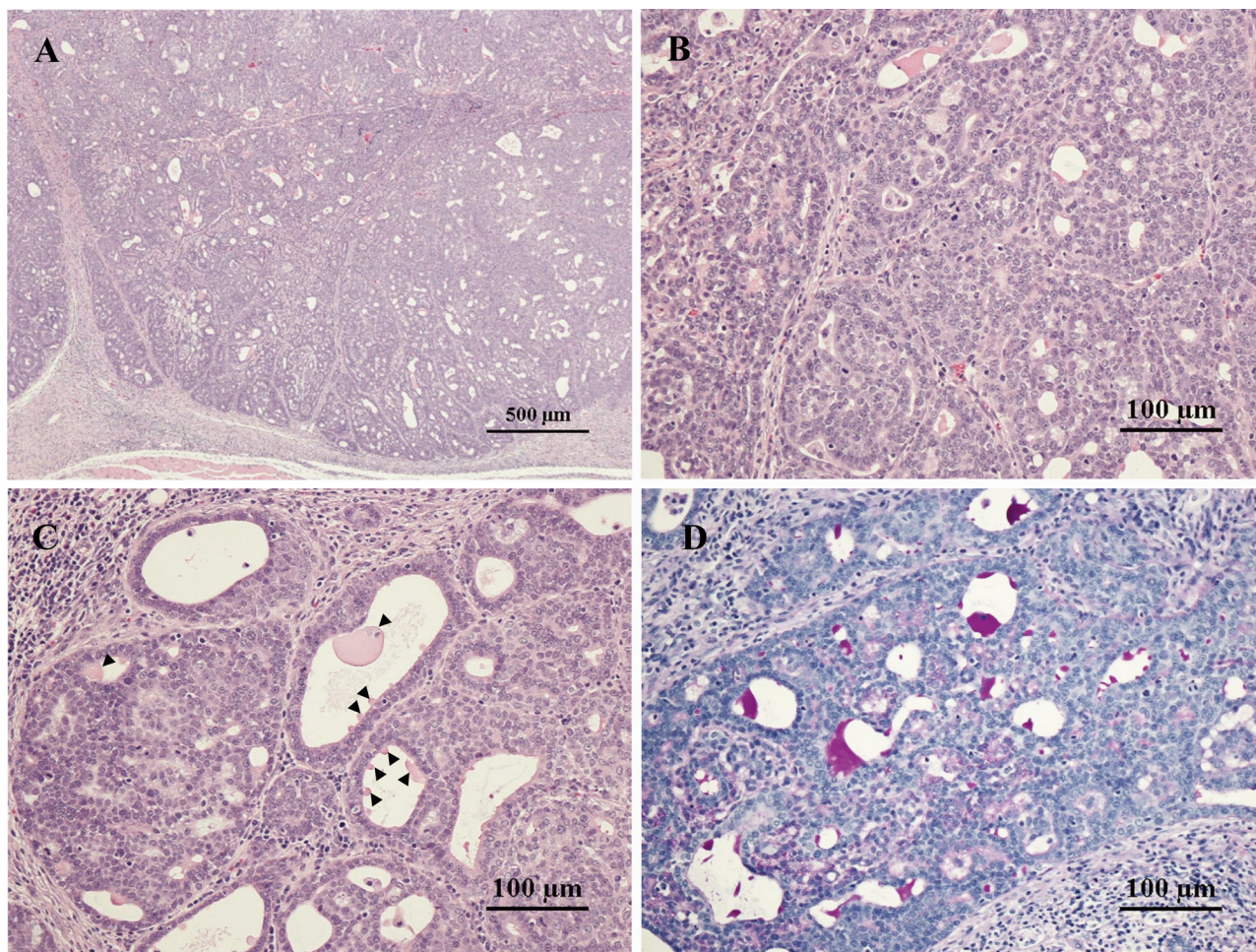


Fig. 1. Histopathological appearance of the tumor. H.E. stain (A–C) and PAS stain (D). A. The tumor was surrounded by a thick capsule, and the tumor was composed of various glandular structures with some acinus or ducts distended with secretion. B. Neoplastic epithelial cells arranged in a solid sheet structure with the formation of secondary lumina (comedo-cribriform pattern) (higher magnification of A). C. Several layers of epithelial cell-lined tubules, glands (tubular pattern) and eosinophilic secreted material were present in the lumen (arrowheads). D. Secreted material was positive for PAS staining.

Table 1. Results of Immunoreactivities

		CK8	CK18	ER- α
Mass	Neoplastic epithelial cells	+	+	+
Normal part				
Mammary gland	Acinar cell	+	+	+
	Lactiferous epithelium	+	+	+
Parotid gland	Acinar cell	–	–	–
	Intercalated ductal cell	–	+	–
	Striated ductal cell	–	+	–
	Excretory ductal cell	+	+	–

of round epithelial cells arranged in glandular structures, including tubular and comedo-cribriform structures and combinations of the two. (Fig. 1A). Although the majority of the tumor showed a comedo-cribriform pattern with multi-layered epithelial cells lining the duct and forming secondary lumina (Fig. 1B), some glands showed a tubular pattern with several layers of epithelial cells lining the tubules (Fig. 1C), and eosinophilic secreted material was present in the lumen (Fig. 1C, arrowheads). This secreted material stained

positive for PAS (Fig. 1D). Neoplastic epithelial cells had basophilic cytoplasm, round to oval nuclei and numerous mitotic structures. Additionally, lymphocyte infiltration and necrosis were observed, but there were no hemorrhages in the tumor.

The immunoreactivities of the tumor, the normal mammary gland and the parotid gland are summarized in Table 1. Some neoplastic epithelial cells stained positive for CK8 and CK18 in the cytoplasm and positive for ER- α in the nu-

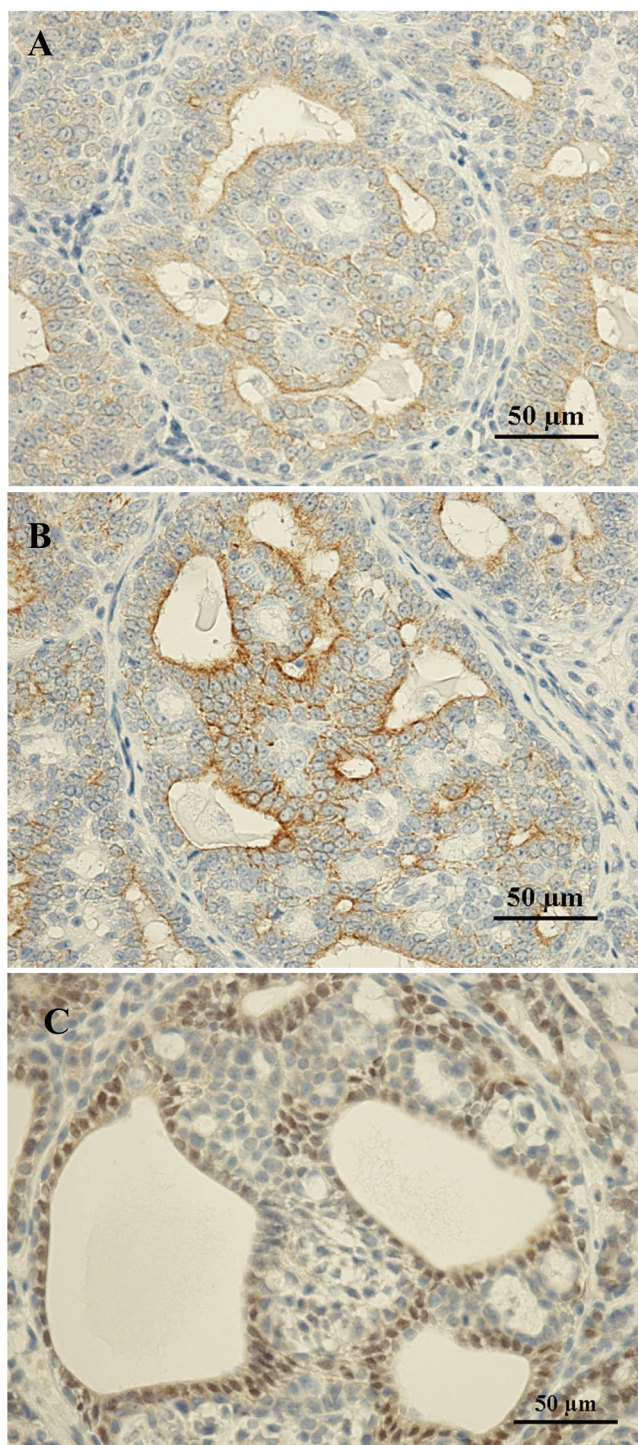


Fig. 2. Immunohistochemical staining of CK8 (A), CK18 (B) and ER- α (C). Neoplastic epithelial cells stained positive for CK8, CK18 and ER- α , particularly neoplastic epithelial cells that formed secondary lumina.

cleus, particularly those that formed secondary lumina (Fig. 2 A–C). Similar to the neoplastic epithelial cells, acinar cells and lactiferous epithelial cells of the normal mammary gland were also positive for these antibodies. In contrast, excretory ductal cells were positive for CK8 and CK18 and

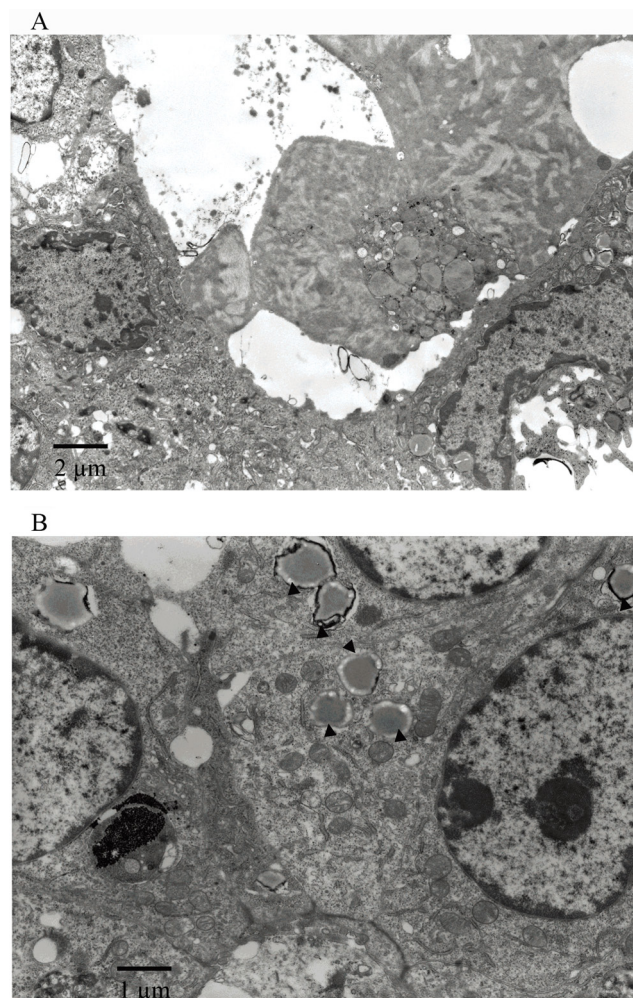


Fig. 3. Electron micrograph of the tumor. A. Secreted material in the lumen. B. Abundant lipid droplets (arrowheads) in the cytoplasm of the neoplastic epithelial cell.

negative for ER- α , but acinar cells were negative for these antibodies in the normal parotid gland.

Examination of the ultrastructure showed the presence of secreted material in the lumen (Fig. 3A). Additionally, abundant lipid droplets enclosed by plasma membranes in the cytoplasm of the neoplastic epithelial cells were observed (Fig. 3B).

In this particular case, neoplastic epithelial cells formed glandular structures, which contained eosinophilic secreted material that was positive for PAS staining in the lumen. Similar secreted material and lipid droplets in the cytoplasm of the neoplastic epithelial cells were observed in the ultrastructure examination. These secreted materials are likely to consist of milk. CK8 and CK18 have been reported to be expressed in epithelial cells of the normal mammary gland^{9, 10} and mammary tumors^{10, 11}. Also, ER- α has been reported to be expressed in epithelial cells of the normal mammary gland and the majority of mammary tumors¹². In this study, neoplastic epithelial cells and acinar cells of the normal mammary gland were also positive for CK8, CK18

and ER- α . From these results, we diagnosed the tumor as a mammary gland adenocarcinoma.

Although adenocarcinomas of the female mammary gland exhibit a wide range of histologic patterns, including papillary, tubular, cribriform and comedo¹³, they are usually diagnosed simply as adenocarcinomas because histological subclassification based on epithelial patterns has not been biologically meaningful in long-term toxicity studies, and furthermore, they often present various combinations of morphological subclassifications^{6, 14}. Likewise, it was considered that our case had a pathognomonic morphological pattern that was characteristic of rat mammary adenocarcinoma. Oishi *et al.* reported that mammary adenocarcinoma occurred in young female SD rat with proliferation of vimentin-positive spindle cells⁸. In this study, immunohistochemical staining for vimentin and α -smooth muscle actin was not performed; however, there was no proliferation of spindle cells like myoepithelial cells in the histopathological and ultrastructural examination.

In general, SD rats have a high incidence of adenocarcinoma relative to F344 rats^{13, 15} and have higher susceptibility to formation of chemical-induced tumors as well¹³. It has been reported that mammary adenocarcinoma occurred after 30 weeks of age in control female SD rats^{6, 16}, and the majority of these cases were fatal¹⁶, similar to cases in old rats⁴. In the literature, the youngest age at which mammary adenocarcinoma was reported in female SD rats was 10 weeks; however, histopathological examination was not performed until 24 weeks of age⁸. To our knowledge, there are no histopathologically diagnosed cases of mammary adenocarcinoma in 12-week-old female SD rats. Our finding, therefore, appears to be a very rare case similar to that previously reported⁸. In conclusion, the case described herein indicates that female SD rats as young as 10–12 weeks of age have sufficient potential to develop spontaneous mammary adenocarcinomas.

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