

Case Report

Spontaneous adenolipoma of the mammary gland in the male F344 rat

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Abstract: A 110-week-old male F344 rat from the high-dose group of a 104-week carcinogenicity study, exhibited a spontaneously occurring subcutaneous mass in the left axilla extending to the chest. Histologically, the mass was well-demarcated from the adjacent mammary tissue and slightly encapsulated without evidence of infiltration into the surrounding tissues. The mass contained both epithelial and adipose components. The epithelial component consisted of ductal structures of various sizes lined by a single layer of flattened to cuboidal epithelial cells with relatively clear or vacuolated cytoplasm. These ductal structures were well-intermingled with an adipose component that consisted of a uniform monomorphic cell population of mature adipocytes. Both cell types were well-differentiated and did not exhibit cellular atypia. Within the mass, fibrous connective tissue was found in the stroma with infiltration of numerous mast cells. Based on these findings, the mass was diagnosed as an adenolipoma of the mammary gland. (DOI: 10.1293/tox.2021-0012; J Toxicol Pathol 2021; 34: 231–234)

Key words: mammary glands, adenolipoma, spontaneous tumor, F344 rat

Among laboratory animals, mixed mammary tumors are fairly common in female dogs but infrequently observed in other species, and histologically consist of at least one epithelial and one mesenchymal component in addition to collagen (e.g., cartilage or bone)¹. In rats, adenolipoma, a tumor consisting of glandular epithelial and mature-appearing adipose tissue, falls into this category^{1,2} and is considered to be an uncommon benign neoplasm of the mammary gland³. There are not many reports on the mammary adenolipomas of rats^{3–7}; only a single case report has described the detailed morphological features of this tumor that occurred in a female Sprague-Dawley rat⁷. Herein, we report the histopathological and immunohistochemical features of a spontaneous adenolipoma of the mammary gland occurring in a male F344 rat.

This male F344/DuCrj rat was purchased at 5 weeks of age from Charles River Japan (Yokohama, Japan), acclimated for 1 week before the start of the experiment, then allocated to a high-dose group in a 104-week feeding carcinogenicity study. The rat was fed a powdered basal diet (CRF-1;

Oriental Yeast Co., Ltd, Tokyo, Japan) 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 and exposed to 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 rats were maintained according to institutional guidelines.

In the latter half of the carcinogenicity study, a subcutaneous mass was detected on palpation and grew from the left axilla to the chest. At the scheduled sacrifice (110 weeks of age), the rat showed no other clinical signs except for bilateral testicular enlargement. No abnormalities, other than the subcutaneous mass or enlarged testes, were detected at necropsy. The subcutaneous mass was irregularly oval, soft, 30 × 20 × 20 mm in size, and well-demarcated from surrounding tissues; its cut surface was almost white.

The subcutaneous mass was fixed in 10% neutral buffered formalin, and the slices were routinely embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Masson's trichrome staining, 0.05% toluidine blue staining (pH 2.5), and immunohistochemistry were performed. For immunohistochemistry, the sections were immersed in 3% hydrogen peroxide (H₂O₂) in methanol to inactivate the endogenous peroxidase activity. The following primary antibodies were used: mouse anti-cytokeratin (monoclonal, AE1/AE3, ready-to-use; Nichirei Corporation, Tokyo, Japan), mouse anti- α -smooth muscle actin (SMA) (monoclonal, 1A4, diluted 1:50; Dako, Glostrup, Denmark),

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and rabbit anti-Ki67 (polyclonal, diluted 1:1000; Abcam, Cambridge, United Kingdom). Antigen retrieval for α -SMA and Ki67 was performed in 10 mM citrate buffer (pH 6.0), and for cytokeratin, it was performed in the Target Retrieval Solution, pH 9 (Dako) using an autoclave for 10 min at 121 °C. After blocking non-specific 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) and 3,3-diaminobenzidine. All sections were

counterstained with hematoxylin.

Histopathological examination revealed that the subcutaneous mass was well-demarcated from the adjacent mammary tissue and slightly encapsulated without evidence of infiltration into the surrounding tissues (Fig. 1a). The normal mammary tissue surrounding the mass exhibited a tubulo-alveolar appearance, whose epithelium was single-layer thick and supported by a layer of myoepithelium and a basement membrane. The mass mainly consisted of an almost equal mixture of tubulo-alveolar epithelial and adi-

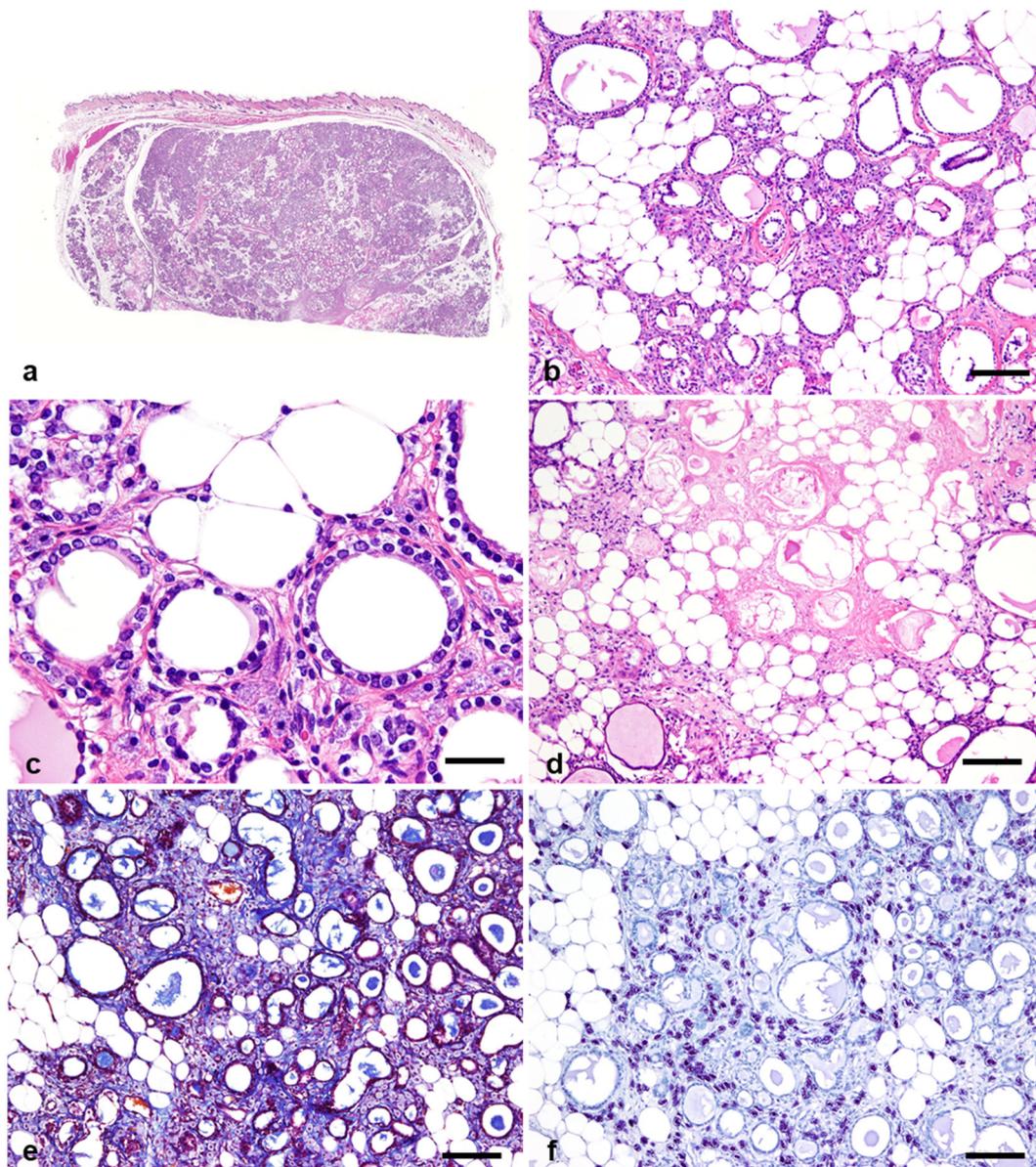


Fig. 1. Histological features of the mass. The mass was well-demarcated from the adjacent mammary tissue and slightly encapsulated (a). The mass was characterized by an almost equal mixture of ductal structures and adipose tissue. Hematoxylin and eosin (H&E) staining. Bar=100 μ m (b). The ductal structures were lined by a single layer of flattened to cuboidal epithelial cells that exhibited relatively clear or vacuolated cytoplasm. Some lumens contained homogeneous eosinophilic materials. Hematoxylin and eosin (H&E) staining. Bar=20 μ m (c). Focal necrosis was sometimes observed. Hematoxylin and eosin (H&E) staining. Bar=100 μ m (d). Masson's trichrome staining depicted the fibrous connective tissue. Bar=100 μ m (e). Numerous mast cells were evenly distributed throughout the fibrous stroma. Toluidine blue staining. Bar=100 μ m (f).

pose components that were well-intermingled in most areas (Fig. 1b). The epithelial component was composed of ductal epithelium lined by a single layer of flattened to cuboidal epithelial cells that exhibited a relatively clear or vacuolated cytoplasm. The lumens were dilated and contained clear or homogenous eosinophilic materials. The epithelial cells were well-differentiated, relatively uniform, and without cellular atypia. Mitotic figures were infrequently encountered. These ductal structures were intermingled with the adipose component, which was spread throughout the mass without pressure on the other components. The adipose component was composed of a uniformly monomorphic cell population of mature adipocytes that had a large, clear vacuolar space with the nucleus compressed against the cell membrane (Fig. 1c). Necrotic areas were sometimes observed within the mass and were characterized by the ghost-like outlines of necrotic adipocytes and epithelial cells (Fig. 1d). Fibrous connective tissue consisting of well-differentiated fibroblasts and collagen was evenly distributed around the ductal epithelium without any atypical features (Fig. 1e) and was especially found in necrotic areas. Numerous degranulated mast cells were uniformly present in the stroma and they did not exhibit cellular atypia or mitotic figures (Fig. 1f). Immunohistochemically, the ductal epithelium was positive for cytokeratin (Fig. 2a). At the basal portion of the epithelium, α -SMA-positive cells were presumed to represent the myoepithelial lining of the ductal structures (Fig. 2b). Within the lesion, proliferating Ki67-positive epithelium was infrequently encountered, and no Ki67-positive adipocytes were detected (Fig. 2c). No noteworthy histopathological findings were detected in other organs and tissues except for the testes, where bilateral Leydig cell tumors were found.

Based on these findings, the histopathological diagnosis of this subcutaneous mass was interpreted as an adenolipoma of the mammary gland. In this carcinogenicity study, although mammary gland tumors, including fibroadenoma, adenoma, and adenocarcinoma, were detected sporadically, no effects of test chemical treatment on the incidence of mammary gland tumors were observed in any group. Morphological features of the present adenolipoma occurring in

a male F344 rat corresponded well with those of the adenolipoma previously described in a female Sprague-Dawley rat, which also consisted of an equal mixture of glandular and adipose tissue elements⁷. Although the differential diagnoses by histopathological examination must include mammary fibroadenoma and adenoma, these tumors are reported to lack significant numbers of intratumoral adipocytes⁸. Thus, adenolipoma can be diagnosed as a benign mammary tumor by detecting the epithelial and adipose tissue components that lack features of malignancy, although necrotic areas were sometimes observed within the mass in the present case. In the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice (INHAND) proposal, adenolipoma is described as a type of benign mixed mammary tumor consisting of uniform epithelial ductules or alveoli and mature adipose tissue². Therefore, the present case would be considered as a "benign mixed mammary tumor, adenolipoma type" in the nomenclature according to the INHAND proposal.

In addition, two unique histopathological features of the adenolipoma of the mammary gland were observed. One feature is the morphology of the epithelial components of the mass. Generally, the mammary glands of male rats show a lobulo-alveolar arrangement of the epithelium, differing from the tubulo-alveolar arrangement observed in female rats⁹. Thus, the glandular tissue in adult male rats is generally arranged in relatively large and contiguous lobular groups of foamy cells that often lack a distinct tubular or ductal appearance². However, the epithelial component of the present mass had a tubulo-alveolar arrangement resembling the morphological appearance generally observed in the mammary gland of female rats. Moreover, the surrounding normal mammary glands also had the tubulo-alveolar arrangement characteristic of female rats. Imbalance of mammatrophic hormones, such as prolactin, estrogens, and androgens can result in the conversion of the mammary glands of male and female rats to resemble the morphological appearance of the opposite sex, and thus mammary gland morphology in the male rat will be altered to that seen in the female rat if androgens are eliminated². In the present

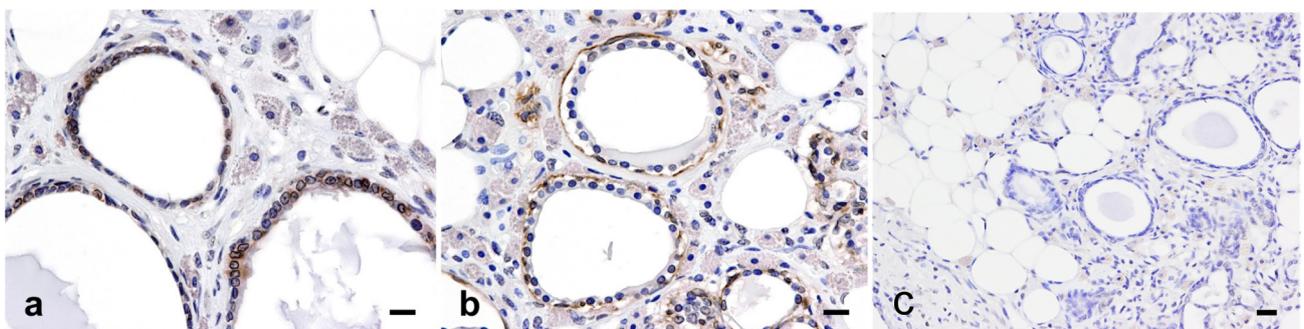


Fig. 2. Immunohistochemical findings of the tumor. The epithelial lining was positive for cytokeratin. Bar=10 μ m (a). The ducts were surrounded by α -smooth muscle actin (α -SMA)-positive cells. Bar=10 μ m (b). Ki67-positive cells were infrequently encountered in the epithelium, and there was no Ki67 reactivity detected in adipocytes. Bar=20 μ m (c).

case, the bilateral testes were completely occupied by Leydig cell tumors, which might have resulted in the exhaustion of normal endocrine functions of the testes. Although this phenomenon has not been clearly reported in rats, several case reports of Leydig cell tumors with gynecomastia and hormonal abnormalities have been reported in humans^{10, 11}. This could result in the conversion of the normal male mammary gland morphology to the tubulo-alveolar morphology, characteristic of female rats. Another characteristic feature of this adenolipoma is the infiltration of numerous mast cells within the stroma. This finding is also described in a previous study of the female Sprague-Dawley rat⁷. Mast cells contribute to the stromal microenvironment in the mammary gland by promoting cell proliferation during terminal end bud formation and duct branching in the growing mammary gland¹². Adenolipomas are categorized as mixed mammary tumors that consist of mammary epithelium and mesenchymal cells². Therefore, mast cells in the stroma might be included in the mesenchymal component of mixed mammary tumors, as they are considered to be essential components of the stromal microenvironment in the mammary gland¹². However, further case studies with more histopathological information will be needed to determine the involvement of mast cells in the pathogenesis of adenolipoma of the mammary gland.

The information presented in this case report could be used to increase awareness about this lesion and to accurately interpret its pathogenesis.

Disclosure of Potential Conflicts of Interest: The authors declare that there are no conflicts of interest.

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