

Case Report

A spontaneous myoepithelial carcinoma in the mammary gland of an aged female ICR (CD-1) mouse

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Abstract: We report a female Crlj:CD1(ICR) mouse with a spontaneous mammary gland tumor composed of biphasic tumor cells, i.e., epithelioid and spindle-shaped myoepithelial cells. Macroscopically, a subcutaneous mass, approximately 3 cm in diameter was found in the lumbodorsal region. Histopathologically, the epithelioid cells proliferated in an alveolar or nest-like growth pattern, occasionally forming glandular-like structures. On the other hand, the spindle-shaped cells proliferated in a sarcomatous pattern. Normal mammary gland was observed in the vicinity of the tumor. Both types of tumor cells showed immunoreactivity for cytokeratin (wide spectrum screening), vimentin, S100, and p63. In addition, the epithelioid cells and spindle-shaped cells were immunopositive for glial fibrillary acidic protein and smooth muscle actin, respectively. Moderate atypia, high proliferative activity, massive necrosis, and partial infiltration to the surrounding tissues were also observed. We made a diagnosis of myoepithelial carcinoma, which is extremely rare in ICR mice. (DOI: 10.1293/tox.2016-0080; J Toxicol Pathol 2017; 30: 245–250)

Key words: ICR mouse, mammary gland tumors, myoepithelial carcinoma, myoepithelial cells, spontaneous tumor

Mammary gland tumors are widely seen in a variety of animal species and generally subdivided into simple or complex tumors by basically relying on the presence of glandular epithelial cells, myoepithelial cells, or both cells^{1,2}. In most mammary tumors, varying proportions of neoplastic glandular epithelial cells and myoepithelial cells or only neoplastic glandular epithelial cells proliferate in association with increased interstitial connective tissues. On the other hand, a tumor in which only neoplastic myoepithelial cells proliferate is known as a myoepithelioma^{1,3}, and this type of tumor is very rare in domestic and companion animals and humans⁴⁻⁷. In rodents, spontaneous myoepithelioma is often found in some strains of mice, such as BALB/c and Strain A³. However, in ICR mice, which are widely used in carcinogenicity studies of chemicals, the incidence is extremely rare; only one case has been reported, and it occurred in a female Icr:Ha(ICR) mouse⁸. Among myoepitheliomas composed of benign and malignant tumors, myoepithelial

carcinoma (malignant myoepithelioma) is uncommon and described as a tumor that has morphological features similar to carcinosarcoma^{3,4}. Detailed information on its histological and immunohistochemical findings is very scarce in rodents. In the present case report, we describe a female ICR mouse with a spontaneous myoepithelial carcinoma composed of neoplastic proliferation of biphasic cells, i.e., epithelioid and spindle-shaped myoepithelial cells, possibly derived from the mammary gland.

The affected animal was a female specific pathogen-free ICR [Crlj:CD1(ICR)] mouse purchased at 4 weeks of age from Charles River Laboratories Japan Inc. (Kanagawa, Japan) and allocated to a control group in a feeding carcinogenicity study for a period of 18 months. The mouse was housed in a stainless steel wire mesh cage in a barrier-sustained animal room with the environment controlled as follows: temperature, 22 ± 2°C; humidity, 50% ± 20%; ventilation, 10 times or more per hour; and illumination, 12 hours/day. It was given a commercial diet (MF Mash, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*, and it was handled during the study in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science (JALAS)⁹ and with the Code of Ethics for Animal Experimentation of the Institute of Environmental Toxicology.

In the affected female mouse, a subcutaneous mass approximately 1 cm in diameter was found in the lumbodorsal

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region during a clinical examination at 80 weeks of age, and it was approximately 3 cm in diameter at 83 weeks of age, at which time the animal was subjected to scheduled terminal necropsy in the carcinogenicity study. At necropsy, the subcutaneous mass was approximately $3 \times 3 \times 2$ cm in size, and on its cut surface, it was pale brown in color and showed a solid appearance with extensive necrosis in the central portion. The macroscopic findings other than the mass included swelling of the skin in the dorsal neck region, enlargement of the spleen, a mass (approximately 2 cm in diameter) in the spleen, enlargement of the systemic lymph nodes, and cystic ovarian bursae of the bilateral ovaries.

All organs and tissues including the mass were fixed in 10% neutral-buffered formalin, embedded in paraffin wax, sectioned at a thickness of 5 μ m, and stained with hematoxylin and eosin (H&E). Sections from the mass were also subjected to periodic acid-Schiff (PAS) staining and immunohistochemistry. The primary antibodies used for immunohistochemistry were as follows: rabbit anti-cow cytokeratin, wide spectrum screening (fully diluted, Dako, Glostrup, Denmark), anti-cow glial fibrillary acidic protein (GFAP) (fully diluted, Dako), anti-chicken desmin (fully diluted, Dako), anti-cow S100 (1:400, Dako), anti-human chromogranin A (1:100, Dako), anti-human synaptophysin (1:10, Dako) and anti-mouse laminin (1:100, Sigma-Aldrich, St. Louis, Mo, USA), mouse anti-human p63 (1:100, Dako), anti-human smooth muscle actin (SMA) (1:100, Dako) and anti-rat proliferating nuclear cell antigen (PCNA) (1:1,000, Dako), and guinea pig anti-calf vimentin (1:50, Progen Biotechnik, Heidelberg, Germany). Antigen retrieval was performed in 0.1 M citrate buffer (pH 6.0) using an autoclave at 121°C for 5 min, except for laminin antibody, which was performed by 0.4 mg/ml proteinase K (Dako) digestion at room temperature for 15 min. An EnVision+ System-HRP anti-rabbit (Dako), EnVision+ System-HRP anti-mouse (Dako), or Biotinylated goat anti-guinea pig IgG (Vector Laboratories, Burlingame, CA, USA) was used as the secondary antibody. The immunohistochemical products were visualized using the substrate 3,3-diaminobenzidine and counterstained with hematoxylin. Sections stained without primary antibodies were used as negative controls. The cross-reactivity of the primary antibodies used in the present study with mouse antigens was confirmed by their data sheets, asking their suppliers, or previous reports^{10, 11}. In addition, cross-reactivity was also confirmed in normal internal tissues or cells, such as nerve fibers, smooth muscle layers, neuroendocrine cells, and mammary glands (glandular epithelial cells and myoepithelial cells).

Histopathologically, the mass was demarcated by peripheral dense connective tissues and mainly localized in the subcutaneous tissue. It was composed of proliferated biphasic tumor cells, i.e., epithelioid cells and spindle-shaped cells (Fig. 1A). The epithelioid cells had relatively round nuclei and abundant eosinophilic cytoplasm and had proliferated in an alveolar or nest-like growth pattern, often being islands demarcated by thin interstitial connective tissues. Glandular-like structures composed by these tumor cells

were also occasionally observed (Fig. 1B). Massive necrosis was noted in the central portion of some of alveolar growth areas. Each island of alveolar or nest-like growth area was incompletely surrounded by a basement membrane that was immunohistochemically positive for laminin and showed a positive PAS reaction. No glandular-like structures surrounded by a basement membrane were observed. The spindle-shaped tumor cells possessed short elongated nuclei and poorly demarcated eosinophilic cytoplasm and proliferated in a sarcomatous pattern. Multinucleated cells were sometimes observed among the spindle-shaped cells (Fig. 1C). The spindle-shaped cells arbitrarily increased between epithelioid islands and sometimes mixed with abundant staghorn-like capillary vessels. A basement membrane was not seen in the spindle-shaped cell-proliferating area. Both types of tumor cells showed moderate atypia of nuclei with a number of mitotic figures and partially infiltrated into the surrounding adipose tissue. Although apparent continuity to the tumor was not clear, normal acinar and ductal structures of the mammary gland were observed in the vicinity of the tumor. Regarding changes other than the subcutaneous tumor, systemic malignant lymphoma was found as a histopathological finding corresponding to macroscopic lesions of the skin in the dorsal neck region, the spleen, and the systemic lymph nodes. Neither the lymphoma nor the other histopathological lesions were considered to be directly related to the subcutaneous tumor in the lumbodorsal region. No metastatic tumor cells of the subcutaneous tumor were detected in any organs or tissues.

The results of immunohistochemistry are shown in Table 1 and Fig. 2 (Fig. 2A–2G). The epithelioid cells were positive for cytokeratin (wide spectrum screening), S100, GFAP, p63 (in a limited population of the tumor cells), and vimentin (in a limited population of the tumor cells). The epithelioid cells composing the glandular-like structures showed similar immunoreactivities but were negative for p63. The spindle-shaped cells were positive for vimentin, S100, SMA, and cytokeratin (wide spectrum screening) (in a limited population of the tumor cells) and weakly positive for p63 (in a limited population of the tumor cells). Both types of tumor cells were completely negative for desmin, chromogranin A, and synaptophysin. High proliferative activity was confirmed by the immunoreaction for PCNA in both types of tumor cells (PCNA labeling index: 34.8% at a high power view). The multinucleated cells observed in the spindle-shaped cell-proliferating area were positive for vimentin.

In contrast to common types of complex mammary gland tumor, the present case was uniquely characterized by biphasic tumor cells, i.e., atypical epithelioid cells and spindle-shaped cells. The epithelioid tumor cells appeared to be similar to glandular epithelial cells with occasional formation of glandular-like structures. In addition, these cells were immunopositive for cytokeratin (wide spectrum screening) and surrounded by PAS- and laminin-positive basement membranes. However, the epithelioid tumor cells were immunopositive for S100 and vimentin, suggesting that the tu-

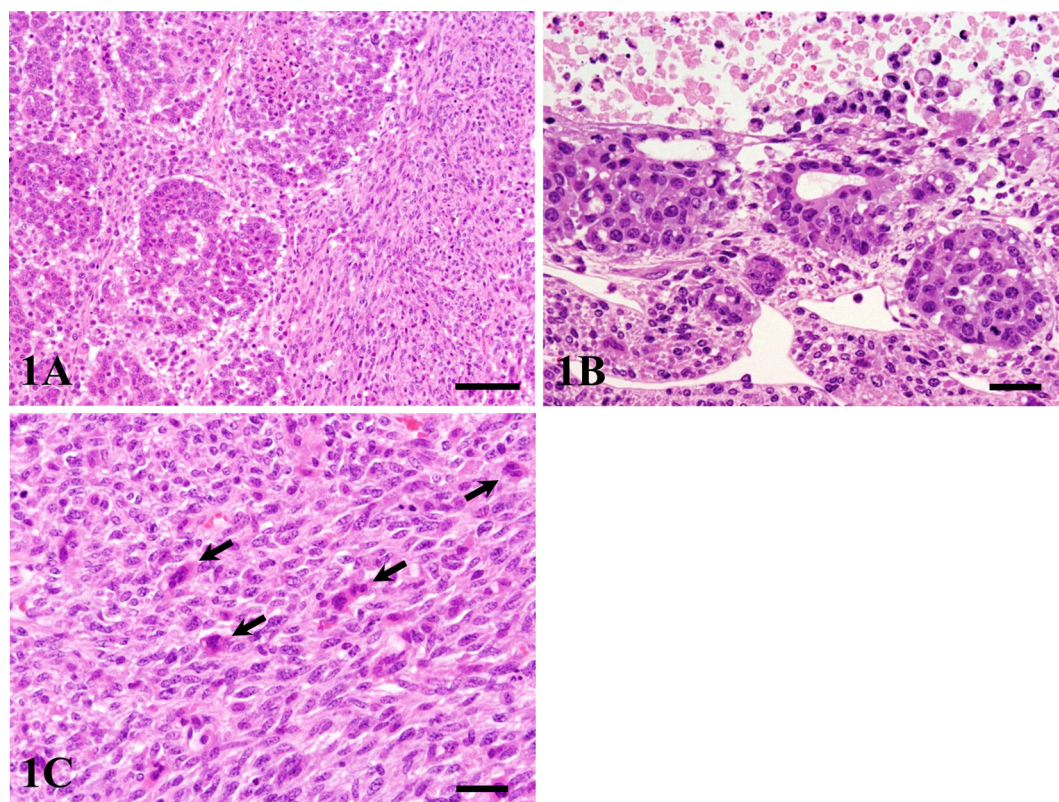


Fig. 1. Histopathological findings of the subcutaneous tumor in the lumbodorsal region. (A) Biphasic proliferation of epithelioid cells in an alveolar or nest-like growth pattern and spindle-shaped cells in a sarcomatous growth pattern. The epithelioid cells had relatively abundant eosinophilic cytoplasm with round to ovoid nuclei, while spindle-shaped cells had ill-defined eosinophilic cytoplasm with spindle-shaped nuclei. (B) Glandular-like structures composed of epithelioid cells. (C) Multinucleated cells (arrows) were sometimes observed and were mixed in with the spindle-shaped cell-proliferating area. H&E. Bar = 50 μ m (A), 20 μ m (B, C).

Table 1. Results of Immunohistochemical Examination of the Tumor Cells

Markers	Types of tumor cells	
	Epithelioid cells	Spindle-shaped cells
PCNA	+	+
S100	+	+
Cytokeratin (wide spectrum screening)	+	+ (limited population)
Vimentin	+ (limited population)	+
p63	+ (limited population)	\pm (limited population)
GFAP	+	-
SMA	-	+
Desmin	-	-
Chromogranin A	-	-
Synaptophysin	-	-

Criteria for grading: +, positive; \pm , weakly positive; and -, negative.

mor cells should be categorized as nonglandular epithelial cells. Furthermore, the tumor cells reacted with p63, which is a reliable marker of myoepithelial tumors¹. The glandular-like structures were negative for p63, but they were positive for both cytokeratin (wide spectrum screening) and vimentin. In addition, no apparent neoplastic glands surrounded by a basement membrane were observed in any sites of the tumor. From these results, it was considered that the epithelioid cells were derived from myoepithelial cells of the

mammary gland. In a previous investigation of the human mammary gland^{5, 12}, a minor subpopulation of normal myoepithelial cells and neoplastic myoepithelial cells of some tumors were immunopositive for GFAP. Therefore, the immunopositivity for GFAP in the present case might support it being of myoepithelial origin. The spindle-shaped tumor cells also shared a common property with myoepithelial cells, i.e., an immunopositive reaction for cytokeratin (wide spectrum screening), p63, vimentin, and S100. Therefore,

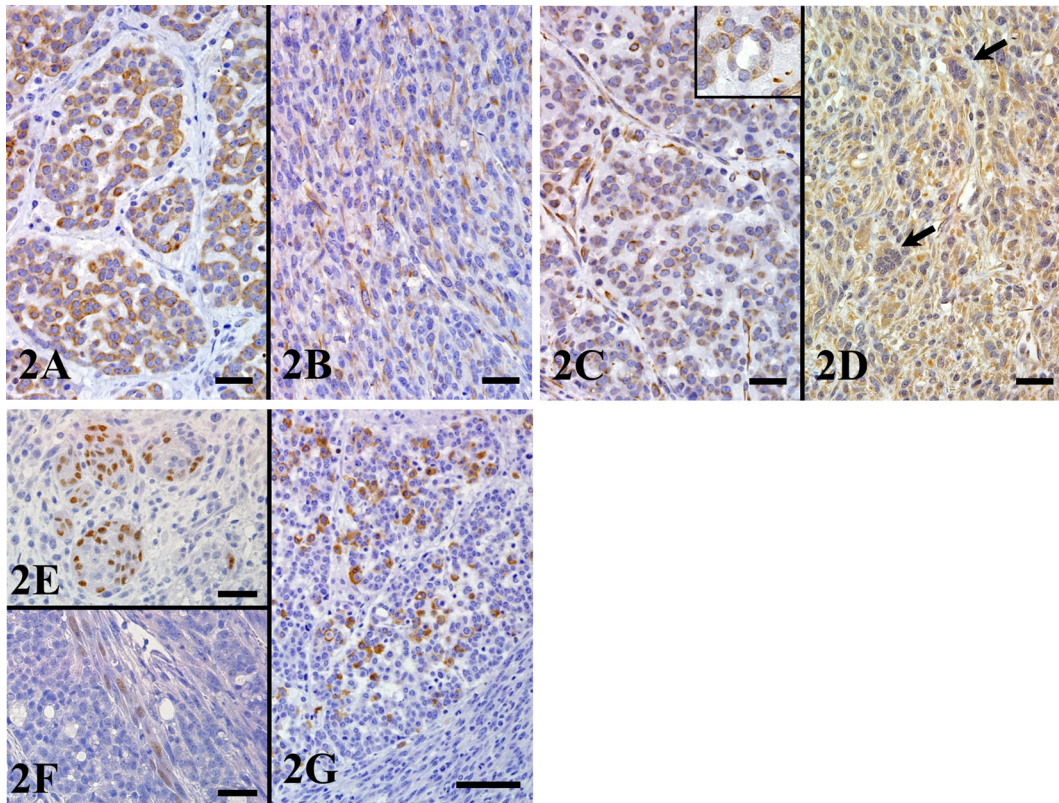


Fig. 2. Immunohistochemical findings of epithelioid cells and spindle-shaped cells of the tumor. The epithelioid cells and spindle-shaped cells were positive (A) and positive in a limited population (B) for cytokeratin (wide spectrum screening), respectively. The epithelioid cells and spindle-shaped cells were positive in a limited population (C) and positive (D) for vimentin, respectively. The glandular-like structures composed by the epithelioid cells (inset in C) and multinucleated cells among the spindle-shaped cells (arrows in D) were also positive for vimentin. Although in a limited population, the epithelioid cells and spindle-shaped cells were positive (E) and weakly positive (F) for p63, respectively. GFAP was positive in only epithelioid cells (G). Bar = 20 μ m (A–F), 50 μ m (G).

both the epithelioid and spindle-shaped cells were considered to be commonly derived from myoepithelial progenitor cells of the mammary gland. In the present case, multinucleated cells were observed in the spindle-shaped cell-proliferating area. They were positive for only vimentin and immunohistochemically did not show any specific direction of differentiation. Multinucleated cells are frequently seen in association with various malignant neoplasms, although the mechanism of neoplastic multinucleation remains unknown. It was considered that the multinucleated cells found in the present case were formed by either cell-cell fusion or acytokinetic cell division, as with neoplastic multinucleation, which is sometimes seen in other malignant tumors.

In a previous report of inbred laboratory mice⁴, myoepithelial cells of the normal mammary gland were immunopositive for SMA, and this was also seen in the normal mammary glands in the present case. However, neoplastic myoepithelial cells were immunonegative for SMA, and this result was different from that of the spindle-shaped cells in the present case. In a previous report, the neoplastic myoepithelial cells composing a mammary gland adenomyoepithelioma were immunopositive for SMA¹³. In the salivary gland, normal myoepithelial cells were immunopositive for

it, but the immunoreactivity of neoplastic myoepithelial cells varied between cases^{4, 14}. Therefore, the SMA immunoreactivity in myoepithelioma derived from the mammary gland might also vary in individual cases. For p63, a very weakly positive reaction was detected in spindle-shaped cells, while a more strongly positive reaction was found in epithelioid cells. Myoepithelial differentiation as indexed by p63 and SMA is complicated in the canine mammary myoepithelial lineage¹⁵. It is hypothesized that progenitor cells that are immunopositive for p63 but not for SMA can differentiate into intermediary myoepithelial cells that are immunopositive for both p63 and SMA and terminally differentiate into myoepithelial cells that are immunopositive for SMA but not for p63¹⁵. By analyzing immunohistochemistry in canine mammary gland tumors, the neoplastic myoepithelial cells of a complex carcinoma appeared to be differentiated myoepithelial cells, while those of a carcinoma-and-malignant myoepithelioma appeared to be undifferentiated myoepithelial cells¹⁵. The morphology, growth pattern, and immunohistochemical properties of neoplastic myoepithelial cells of a canine complex carcinoma and those of a carcinoma-and-malignant myoepithelioma could be similar to those of spindle-shaped cells and epithelioid cells in the

present case, respectively. Therefore, in the present case, the neoplastic spindle-shaped cells might have differentiated into myoepithelial cells more than the neoplastic epithelioid myoepithelial cells did. This hypothesis is supported by the finding indicating that the murine benign myoepithelioma is composed of spindle cells with no cellular atypia³, which might correspond to well-differentiated myoepithelial cells.

In the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) for experimental rodents, mammary gland tumors are classified as adenomas, adenocarcinomas, benign mixed tumors (fibroadenomas), carcinosarcomas, and adeomyoepitheliomas². Adenomas and adenocarcinomas, known as simple adenomas or adenocarcinomas, are composed of neoplastic proliferations of glandular epithelial cells with or without malignancy and scant interstitial connective tissues. The other three tumors are characterized by proliferations of biphasic cells as seen in the present case. However, benign mixed tumors contain osseous and cartilage tissues in addition to proliferating glandular epithelial cells and myoepithelial cells, and carcinosarcomas are composed of high-grade neoplastic proliferations of both glandular epithelial cells and non-myoepithelial mesenchymal cells. Adenomyoepitheliomas, which are formally called complex adenomas or carcinomas in dogs^{6, 16}, are composed of neoplastic proliferations of glandular epithelial cells and myoepithelial cells. A spontaneous adenomyoepithelioma in the mammary gland was reported in a one-year-old female C57BL/6 mouse that had tubular or cord-like structures of neoplastic epithelial cells mixed with dense bundles of neoplastic myoepithelial cells with a clear and mucinous matrix¹³. We can exclude these tumors in diagnosing the present case because it did not contain neoplastic glandular epithelial tumor cells. A myoepithelioma composed of only neoplastic myoepithelial cells is not categorized as a mammary gland tumor in the INHAND. Nevertheless, myoepithelioma is the most suitable diagnosis for the present case. The present case should be diagnosed as a case of myoepithelial carcinoma because moderate atypia and high proliferative activity of tumor cells, massive necrosis, and partial infiltration of tumor cells into the surrounding tissue were evident and were similar to the findings in previous cases of myoepithelial carcinoma in mice that were induced with 7,12-dimethylbenzene¹⁷. Spontaneous myoepithelial carcinoma is uncommon in mice^{3, 4}, and knowledge concerning its immunohistochemical properties is extremely limited¹⁴. The present case report provides detailed histological and immunohistochemical properties of murine myoepithelial carcinoma and useful insights for understanding rodent mammary gland tumors derived from the myoepithelial lineage.

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