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Case Report

Spontaneous malignant myoepithelioma of the maxillary gland in a young adult male BALB/c F1 hybrid mouse

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Abstract: A spontaneous tumor was observed in the maxillary gland of a 12-week-old male BALB/c F1 hybrid mouse. Histopathologically, the tumor invaded both the nasal cavity and maxillary sinus, and ethmoturbinates were destroyed. The majority of the tumor cells had oval nuclei with eosinophilic and vacuolar cytoplasm. Numerous mitotic figures and necrotic foci were also observed throughout the tumor. Immunohistochemically, almost all of the tumor cells were strongly positive for cytokeratin (WSS) and alpha-smooth muscle actin. However, tumor cells were negative for cytokeratin (CAM 5.2), suggesting that these tumor cells originated from myoepithelial cells. The present tumor was diagnosed as a malignant myoepithelioma of the maxillary gland. This is the first report describing spontaneous malignant myoepithelioma in the maxillary gland of a young adult BALB/c F1 hybrid mouse. (DOI: 10.1293/tox.2015-0032; J Toxicol Pathol 2016; 29: 111–114)

Key words: malignant myoepithelioma, maxillary gland, BALB/c F1 hybrid mouse

Spontaneous tumors in the salivary glands of different rodents have been reported[1–4]. In inbred mice, it is well known that spontaneous myoepitheliomas in the salivary glands are common, and they are expected to occur in 3–4% of older female BALB/c mice and their F1 hybrids[5]. The maxillary gland is a minor salivary gland located in the posterior dorsal portion in the subepithelial layers of the maxillary sinus of rodents[6]. Tumors in the maxillary gland are rare[7, 8], and only one case of a spontaneous adenocarcinoma with a myoepithelial component has been reported in an older male CD-1 mouse[9]. This report describes a case of spontaneous malignant myoepithelioma of the maxillary gland of a 12-week-old male BALB/c F1 hybrid mouse.

The mouse that developed the tumor was one of three 12-week-old male BALB/c F1 hybrid mice treated with phosphate-buffered saline (PBS) in an experiment. In the experiment, a female BALB/cByJ mouse (CLEA Japan Inc., Tokyo, Japan) mated with a male DO11.10 mouse (The Jackson Laboratory, Bar Harbor, ME, USA), to bear fetal mice of the first filial generation. The genetic background of DO11.10 mice is based on BALB/c mice. All mice were housed individually in plastic cages in an air conditioned room at 24 ± 2°C and 55 ± 5% relative humidity under a 12-h light/dark cycle. They were fed CE-2 sterilized by γ-rays (CLEA Japan Inc.) and tap water *ad libitum*. The animal experiment protocol used in the study was approved by the Institutional Animal Care and Use Committee of the Tokyo University of Agriculture and Technology. No clinical abnormalities were observed in the mouse with the tumor. The mouse was euthanized by cervical dislocation under diethyl ether anesthesia at 12 weeks of age. After necropsy, all sampled organs and tissues including the nose were fixed in 10% phosphate-buffered formalin for histopathological examination. The nasal tissue was decalcified with a mixture of 20% formic acid and 20% formalin for several days and trimmed before embedding in paraffin. Paraffin sections were stained with hematoxylin-eosin (HE). Additional immunohistochemical staining was performed with serial sections. The primary antibodies used in the study are described in Table 1. Immunohistochemical staining of cytokeratin (WSS) was performed with an EnVision™ kit (Dako, Tokyo, Japan), and immunohistochemical staining of cytokeratin (CAM 5.2) and alpha-smooth muscle actin (α-SMA) was performed with a Histofine MOUSESTAIN Kit (Nichirei Co., Ltd., Tokyo, Japan).

At necropsy, no macroscopic abnormalities were observed. Histopathologically, the tumor was incidentally detected in the subepithelial layer of the maxillary sinus (Fig. 1A). The acinus of the maxillary gland adjacent to the tumor was atrophic. The tumor had high cellularity and invaded both the nasal cavity and maxillary sinus (Fig. 1A), and ethmoturbinates were destroyed (Fig. 1B). Multiple necrotic foci were observed throughout the tumor (Fig. 1B and 1C). Most of the tumor cells had pale oval

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Table1. Primary Antibodies Used for Immunohistochemistry

Primary antibody	Dilution	Antigen retrieval
Polyclonal Rabbit Anti-Cytokeratin, Wide Spectrum screening (WSS) code Z0622, Dako	1: 500	Proteinase K (6 min)
Anti-Cytokeratin (CAM5.2) code 349205, Becton, Dickinson and Company (San Jose, USA)	Prediluted	Proteinase K (6 min)
Monoclonal Anti-Human Smooth Muscle Actin Clone 1A4, code M0851, Dako	1:100	Microwave (5 min)

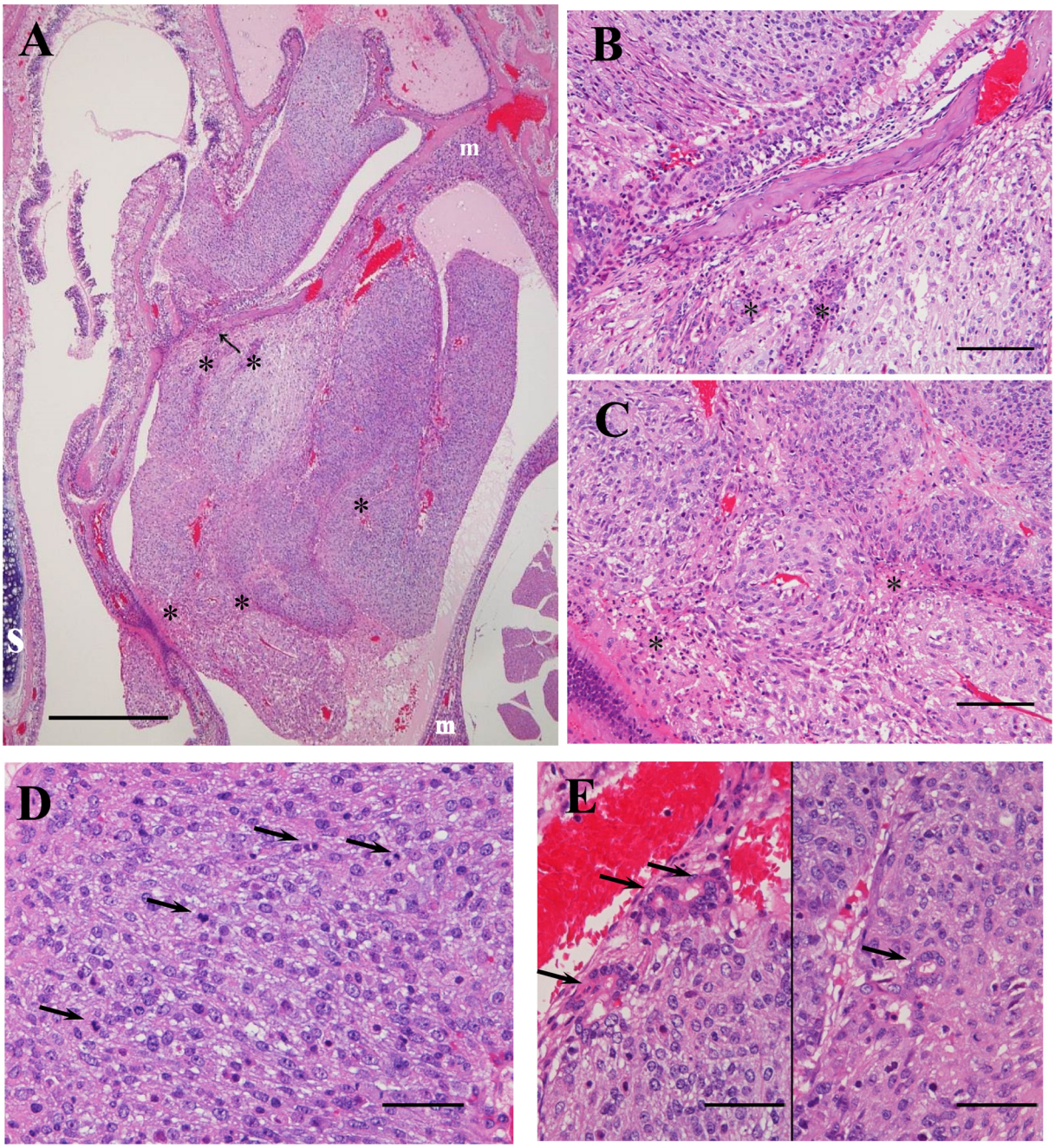

Fig. 1. Histopathological cross section of the nose with HE staining (A–E). A: Section of the tumor from the subepithelial layers of the maxillary sinus. The tumor has invaded both the maxillary sinus and nasal cavity, and there is invasion of ethmoturbinates (arrow) and multiple necrotic foci (asterisks). S, nasal septum; m, maxillary gland. Scale bar: 500 µm. B, C: High magnification of Fig. 1A. The invasion of ethmoturbinates and multiple necrotic foci (asterisks) are observed. Scale bar: 100 µm. D: The tumor cells have oval nuclei, and they contain vacuolar pale eosinophilic cytoplasm. There are numerous mitotic figures (arrows). Scale bar: 50 µm. E: Small duct-like structures are seen (arrows). A few duct-like structures are adjacent to the tumor (left), and another is in the tumor (right). Scale bar: 50 µm.

nuclei containing a few prominent nucleoli, and there was vacuolar pale eosinophilic cytoplasm (Fig. 1D). There were numerous mitotic figures in the tumor (Fig. 1D). A few duct-like structures lined with cuboidal epithelial cells were in or adjacent to the tumor (Fig. 1E). Immunohistochemically, the tumor cells were positive for cytokeratin (WSS) and α-SMA (Fig. 2A and 2C) but negative for cytokeratin (CAM 5.2) (Fig. 2B). The immunohistochemical results were strikingly similar to those in the normal myoepithelial cells (Fig. 2D). Although some cells were negative for α-SMA, it is known that neoplastic myoepithelial cells are occasionally negative for α-SMA[10].

The histopathological and immunohistochemical findings in this case indicated a diagnosis of malignant myoepithelioma. The tumor was mainly present in the subepithelial layer of the maxillary sinus, and no other tumor lesions were observed in other organs or tissues, which supports that this malignant myoepithelioma derived from the maxillary gland. The present tumor was assumed to have occurred spontaneously because the mouse had been treated with PBS only. In the maxillary gland of mice, biphasic tumors have been induced by polyoma virus (PV)[8]. However, it could not be determined whether this mouse was infected with PV. There is the possibility of the tumor being an epithelial-myoepithelial carcinoma, because a few duct-like structures were observed in or adjacent to the tumor. However, the duct-like structures adjacent to the tumor were assumed to be residual ducts that showed a regenerative reaction caused by compression of the tumor. Only a few internal duct-like structures were noted throughout the tumor. In the investigations here, no other internal duct-like structures were observed, even in the other serial sections, and these internal duct-like structures were thought to be regenerative residual ducts as well. This led to the conclusion that the cells of the duct-like structures were not neoplastic cells and that a diagnosis of epithelial-myoepithelial carcinoma was not a possibility. Sundberg *et al.* (1991) reported that myoepitheliomas of inbred laboratory mice did not occur before 107 days of age[11]. In particular, no other case was found before 148 days in BALB/cByJ mice[11]. This makes the present report the first to describe spontaneous malignant myoepithelioma in the maxillary gland of a young adult BALB/c F1 hybrid mouse.

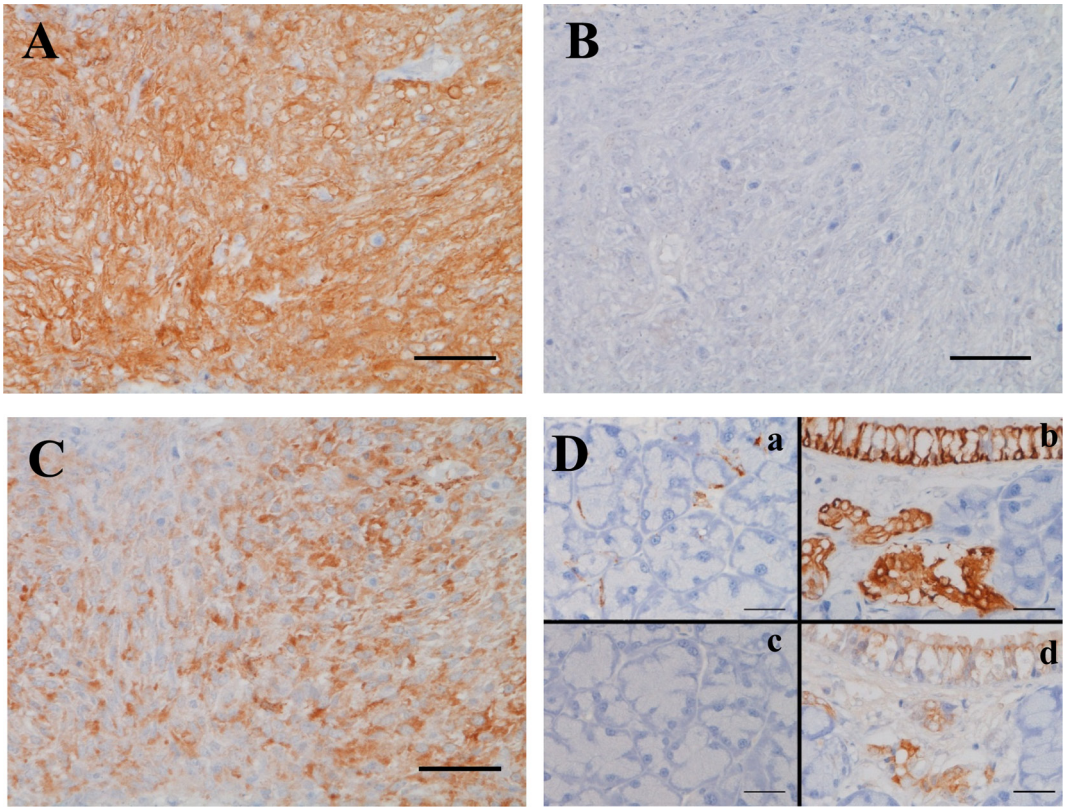

Fig. 2. Immunohistochemistry of cytokeratin (WSS), cytokeratin (CAM 5.2) and α-SMA (A-D). A: Immunohistochemistry of cytokeratin (WSS). Tumor cells are strongly positively stained throughout the tumor. Scale bar: 50 μm. B: Immunohistochemistry of cytokeratin (CAM 2.5). Tumor cells show negative reactions. Scale bar: 50 μm. C: Immunohistochemistry of α-SMA. The majority of the tumor cells are strongly positive for α-SMA throughout the tumor, although some of the tumor cells are α-SMA negative. Scale bar: 50 μm. D: Normal myoepithelial cells of the maxillary gland (a, c) and epithelial cells of the excretory ducts of the maxillary gland (b, d) stained for cytokeratin (WSS) and cytokeratin (CAM 2.5) are shown. Both myoepithelial cells and epithelial cells are positive in the specimen stained for cytokeratin (WSS) (a, b). In the specimen stained for cytokeratin (CAM 2.5), myoepithelial cells are negative, while epithelial cells are positive (c, d). Scale bar: 25 μm.

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