Case Report

A spontaneous basal cell carcinoma with pulmonary metastasis in a 6-week-old SD rat

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Abstract: To our knowledge, this is the first report on basal cell carcinoma with lung metastasis in a rat. A 6-week-old male Sprague-Dawley rat presented ulceration of the oral mucosa with surrounding tumor growth and white nodules in the lung. Microscopically, the mass showed solid, sheet-like growth with a partially lobular pattern and invaded the gingival mucosa, maxilla, and nasal submucosa. The nuclei of tumor cells were round to oval in shape with basophilic cytoplasm and a large number of mitotic figures. The pulmonary nodules were almost identical to the maxillary tumor in histopathological characteristics. Immunohistochemically, tumor cells were positive for cytokeratin, vimentin, PCNA, and p63 and negative for desmin, S-100, and αSMA. Based on these results, we diagnosed the tumor as a maxillary basal cell carcinoma with pulmonary metastasis. (DOI: 10.1293/tox.2017-0016; J Toxicol Pathol 2017; 30: 323–326)

Key words: basal cell carcinoma, pulmonary metastasis, rat

Basal cell carcinoma is rare in rats. Isolated cases (one each) have been reported at 7, 10, and 19 weeks of age[1–3], and 2 cases have been reported at around 87 weeks of age[4]. Basal cell carcinoma shows invasive growth and local tissue destruction, but metastasis is rare. To the authors' knowledge, there is no report of basal cell carcinoma with distant metastasis in rats. Metastasis is rare in humans, and metastasis rates in human patients with basal cell carcinomas range from 0.0028% to 0.55%[5]. To our knowledge, the present case is the first report of basal cell carcinoma with distant metastasis in rats. This case occurred in a 6-week-old male Crl:CD(SD) rat (Charles River Laboratories Japan, Inc.) that presented abnormal respiratory sounds and black stool in the acclimation period of a toxicity study at Shin Nippon Biomedical Laboratories, Ltd. (SNBL). The animal had been individually housed in a stainless steel cage (300 mm [D] × 170 mm [W] × 180 mm [H]) in an animal room maintained under controlled conditions (12 hours/day of artificial light, temperature of 22 ± 3°C, and relative humidity of 30–70%). All animal husbandry procedures were approved by the Animal Care and Use Committee of SNBL and performed in accordance with the animal welfare by-laws of SNBL, which is fully accredited by AAALAC International.

The rat, which had been judged to be moribund, was euthanized by exsanguination after anesthesia by an intraperitoneal injection of sodium pentobarbital solution (6.48 mg/mL, 5 mL/kg, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and submitted for necropsy. The necropsy revealed ulceration of the oral mucosa (5 mm in diameter) in the left hard palate involving the upper left first molar (Fig. 1A). Compression of the palatine rugae around the ulcer was identified and determined to be due to tumor growth, although the tumor was difficult to discern macroscopically. After fixation, the ulcer portion was cut transversely, and a mass was found on the cutting plane. The maxillary bone was found to be protruding into the left orbital cavity after removal of the ipsilateral eyeball. The rat also had 3 white nodules (1 to 2 mm in diameter) in the lungs (Fig. 1B) and dilatation of the small intestine and cecum with a luminal retention of gas and black material. No abnormal region was observed in other organs macroscopically. The maxilla, nasal cavity, lungs, liver, spleen, heart, kidneys, brain, and spinal cord were collected and fixed in 10% neutral buffered formalin, and the maxilla and nasal cavity were decalcified with ethylenediaminetetraacetic acid (EDTA). These tissues were embedded in paraffin, sectioned, and stained with hematoxylin-eosin (HE). The maxillary region was also stained with periodic acid-methenamine silver (PAM). The following 8 kinds of antibodies were used for immunohistochemical stainings: monoclonal anti-cytokeratin (pan keratin) antibody (Clone AE1/AE3, Dako, Japan), monoclonal anti-cytokeratin 5/6 (CK5/6) antibody (Clone D5/16B4, Invitrogen, USA), monoclonal anti-p63 antibody (Clone
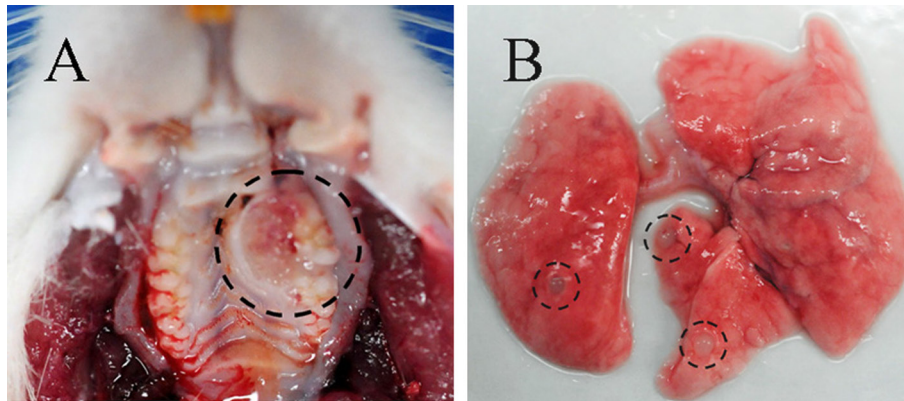
Fig. 1. Macroscopic appearance. (A) Maxillary lesion. The surface was ulcerated. (B) White nodules in the lung.

Y4A3, Acris, Germany), monoclonal anti-PCNA antibody (Clone PC10, Dako, Japan), monoclonal anti-vimentin antibody (Clone V9, Dako, Japan), monoclonal anti-α-SMA antibody (Clone 1A4, Dako, Japan), polyclonal anti-S-100 antibody (Abcam, UK), and polyclonal anti-desmin antibody (Abcam, UK). The maxillary region was stained with all of the above antibodies, and the lung was stained with pan keratin, p63, and vimentin. Deparaffinized sections were incubated with these primary antibodies at room temperature for 60 minutes and then reacted with the peroxidase-labeled secondary antibodies at room temperature for 30 minutes using Histofine Simple Stain MAX-PO (MULTI) (Nichirei, Japan). Positive reactions were visualized with 0.02% 3,3′-diaminobenzidine (DAB), and the sections were counterstained with hematoxylin.

Microscopically, the tumor was widespread in the left maxillary region and invaded the gingival mucosa, the dental pulp of the first upper left molar, the tissue adjacent to the maxilla, and the nasal submucosa. The invading tumor distorted the nasal trabeculae and narrowed the nasal space (Fig. 2A). The solid, sheet-like tumor growth showed a partially lobular pattern (Fig. 2B, 2C, and 2D). Cellular boundaries were indistinct. Tumor cells had round-to-oval nuclei with single or multiple nucleoli and basophilic cytoplasm. A large number of mitotic figures were seen (Fig. 2E). There was no evidence of squamous differentiation or intercellular bridges. Abundant proliferation of stromal spindle cells was also observed. Histopathological characteristics of the pulmonary lesions were identical to those of the maxillary tumor (Fig. 2F).

The results of immunohistochemical staining are summarized in Table 1. Maxillary tumor cells were positive for pan keratin, CK5/6, p63, and PCNA (Fig. 3A, 3B, 3C, and 3D, respectively), and the pulmonary tumor cells were positive for pan keratin and p63 (Fig. 3F and 3G, respectively). Maxillary tumor cells were negative for α-SMA, S-100, and desmin. Both maxillary and pulmonary tumor cells were weakly positive for vimentin (Fig. 3E and 3H). p63 is known to be a marker for basal cells[6], and CK5/6 is used as a marker for squamous cells[7] and basal epithelial cell layers[8]; how-

ever, the present case lacked squamous differentiation such as keratinization and intercellular bridges. The tumor cells were basophilic and resembled basal cells. These results are consistent with basal cell carcinoma.

Basal cell carcinomas originate from epithelial basal cells and are rare malignant tumors in rats, with only a small number of cases reported in the lips, nose, back, inguinal region, or submandibular gland[3, 4, 9]. In the present case, the tumor was considered to have originated at the maxillary region because the tumor was widespread in that area, whereas only small neoplastic nodules were observed in the lung. Basal cell carcinomas have never been reported at this location in rats; however, we could not determine whether the tumor originated in the epithelium of the oral or nasal cavity, since apparent continuity between tumor cells and the olfactory epithelium, nasopharyngeal duct epithelium, olfactory gland, maxillary sinus gland, and squamous epithelium of the hard palate was not observed.

When the nasal cavity was occluded by a nasal tumor, rats showed mouth breathing that resulted in gas retention in the intestinal tract[10]. The abnormal respiratory sounds and intestinal luminal retention of gas and black material in the present case were probably caused by an occlusion of the nasal cavity by tumor invasion and bleeding at the ulcerated hard palate.

The tumor cells in the present case showed numerous mitotic figures and destructive proliferation accompanied by distant metastasis, which is reported to be rare in humans[5]. We diagnosed the tumor to be a highly malignant, spontaneous basal cell carcinoma accompanied by lung metastasis. To the authors' knowledge, this case with lung metastasis is the first such report in rats.
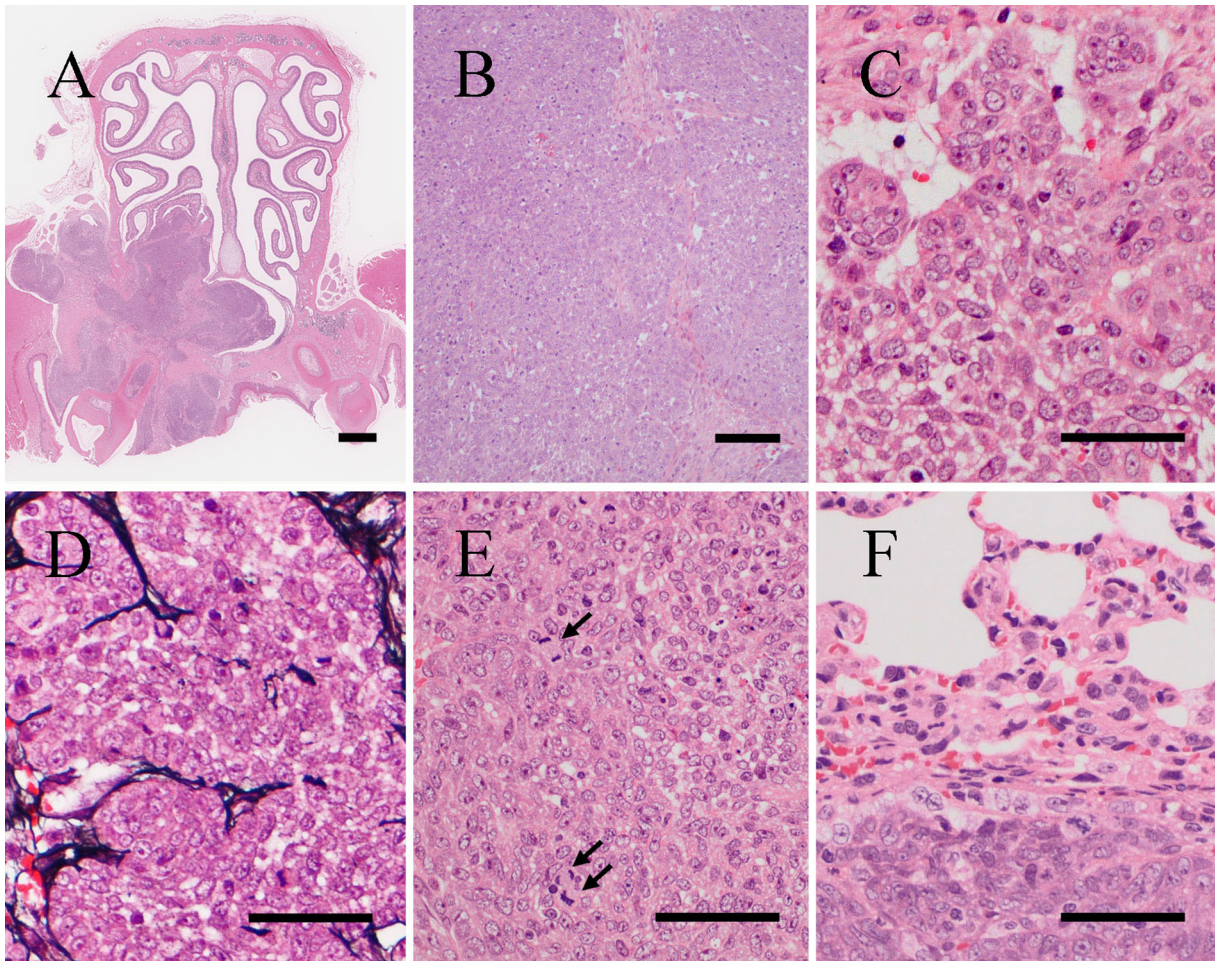
Fig. 2. (A) Maxillary lesion. Tumor cells infiltrated the hard palate and nasopharynx. HE stain. Bar = 1,000 µm. (B) Tumor cells in the maxilla. Tumor cells showed the solid and sheet-like growth under a low-power field. Bar = 100 µm. (C) Tumor cells in the maxilla. Tumor cells showed a pattern of partial lobular proliferation. HE stain Bar = 50 µm. (D) Tumor cells in the maxilla were separated by a thin connective tissue stroma. PAM stain. Bar = 50 µm. (E) Tumor cells in the maxilla contained basophilic cytoplasm, and the nuclei were round to oval in shape with single or multiple nucleoli. Many mitotic figures were seen (arrows). HE stain. Bar = 50 µm. (F) Tumor cells in the lung. The border between nodule and normal lung tissue was clear. The tumor cells in the lung nodules were similar to those in the maxillary lesion (B). HE stain. Bar = 50 µm.

Table 1. Results of Immunohistochemical Staining

| Antibody | Positive cells in references | Maxilla | | Lung |
		Tumor cells	Stromal cells	Tumor cells
Pan keratin	Epithelial	+	−	+
Cytokeratin 5/6	Epithelial (basal and squamous)	+	−	NE
p63	Basal	+	−	+
PCNA	Proliferating	+	±	NE
Vimentin	Mesenchymal	±	+	±
α-SMA	Smooth muscle	−	±	NE
S-100	Neuroectodermal origin	−	−	NE
Desmin	Muscle	−	−	NE

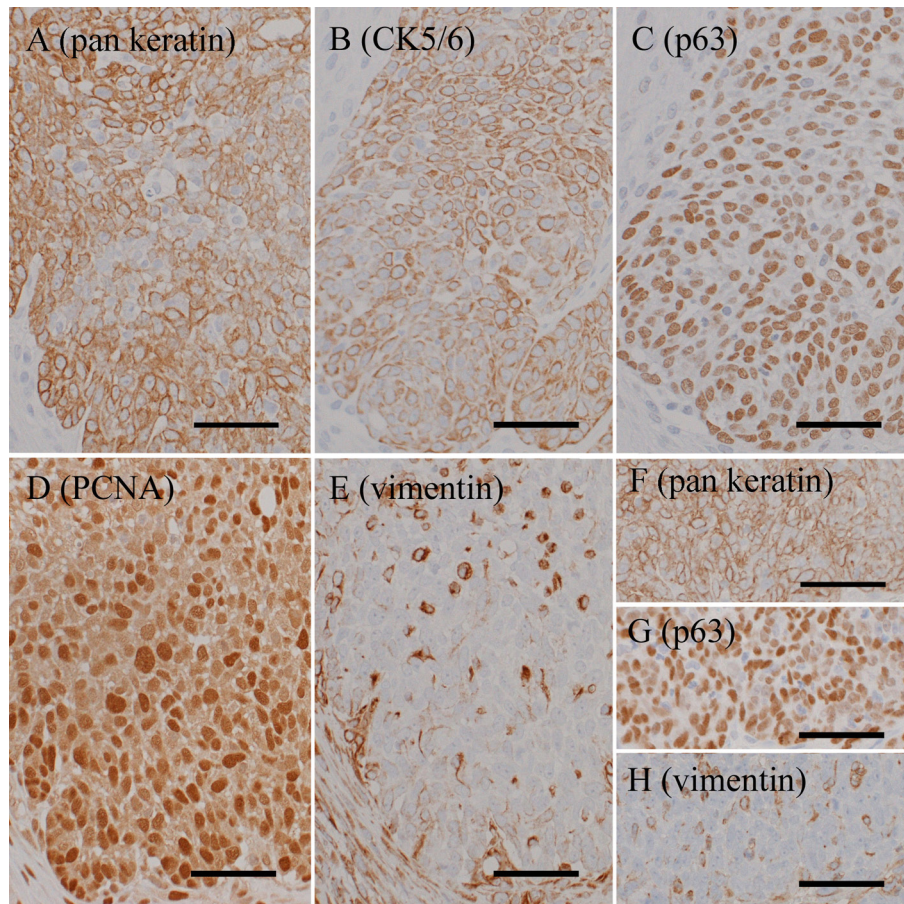+: Positive, ± : Weakly positive, −: Negative, NE: Not examined.

Fig. 3. Immunohistochemical staining of tumor cells. Maxilla: Tumor cells in the maxilla were strongly positive for pan keratin (A), CK5/6 (B), p63 (C), and PCNA (D). Spindle cells around the tumor and some tumor cells were weakly positive for vimentin (E). Lung nodule: Results of staining similar to those for tumor cells in the maxilla. Pan keratin (F), p63 (G), vimentin (H). Bars = 50 μm.

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