Malignant epithelial tumors have frequently been observed in rasH2-Tg mice. Spontaneous hemangiosarcoma and sulfisoxazole-induced adenoma have also been reported in the epididymis of rasH2-Tg mice, but no other epididymal tumor lesions have been reported. In a 26-week carcinogenicity study in rasH2-Tg mice conducted at Shin Nippon Biomedical Laboratories, Ltd (SNBL), primary squamous cell carcinoma was found in the epididymis of a 29-week-old male rasH2-Tg mouse (CLEA Japan, Inc. Tokyo, Japan) in a positive control group treated with N-methyl-N-nitrosourea (MNU; single intraperitoneal administration, 75 mg/kg). Due to the rarity of epithelial tumors originating from the epididymid ducts, the mouse was housed under controlled conditions (12 h light/dark cycle, temperature of 22°C ± 3°C, relative humidity of 50% ± 20%) and were given free access to food (autoclaved CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) at moribundity and was euthanized by exsanguination under isoflurane-induced anesthesia (2.0 to 4.0%, Zoetis Japan Co., Ltd., Tokyo, Japan). Positive reactions were visualized with 3,3′-diaminobenzidine (DAB), and the sections were counterstained with hematoxylin. All animal husbandry procedures were approved by the Animal Care and Use Committee of SNBL and were performed in accordance with the animal welfare bylaws of SNBL, which is fully accredited by AAALAC International.

Twenty-one weeks (Day 147) after the single administration of MNU, the mouse was euthanized by exsanguination under isoflurane-induced anesthesia (2.0 to 4.0%, Zoetis Japan Co., Ltd., Tokyo, Japan) at moribundity and was then submitted for necropsy. At necropsy, a white-grayish mass (15×12×7 mm) was observed in the left caput epididymis (Fig. 1). The mass had not adhered to the surrounding tissue (scrotum or testis). Other macroscopic findings consisted of a white cauliflower-like mass of the scrotal dermis (12×10×7 mm, 1 mass) and the mucosa of the forestomach (2×3×4 mm, 5 masses), swelling of spleen, liver, and lymph nodes (submandibular, axillary, parathymus, hilus, pancre-
aticoduodenal, mesenteric, renal, iliac, inguinal, and popliteal), red cloudy ascites, and pleural effusion.

Histopathologically, the mass was composed of nodular and infiltrative growth lesions (Fig. 2A). The nodular lesion was surrounded by thick fibrous connective tissue that was Masson’s trichrome staining positive (Fig. 2B). In the nodular lesion, polygonal cells proliferated in alveolar or sheeted structures (Fig. 3A, 3C). The alveolar structure was surrounded by argyrophilic fibrous tissue (Fig. 3B). In the alveolar and sheeted structures, the tumor cells had abundant cytoplasm that was both eosinophilic and basophilic, formed well-defined intercellular bridges, and exhibited a cobblestone-like arrangement (Fig. 3C, 3D). Some tumor cells represented a tendency to keratinize (Fig. 3A, 3D), but the formation of cancer pearls was not evident. An irregularly shaped cavity was observed in the alveolar structures. This cavity contained keratin-like material and keratinocytes (Fig. 3D). Several epididymal ducts remained in the nodular lesion (Fig. 3E). These epididymal ducts consisted of normal columnar epithelial and basal cells with no evidence of relation to the tumor cells (Fig. 3E). In the infiltrative growth lesion, spindle-shaped cells proliferated in a trabecular structure (Fig. 3F). In the trabecular structure, the tumor cells did not exhibit keratinization or the formation of distinct intercellular bridges (Fig. 3F). Tumor cells had atypical nuclei, and frequent mitotic figures were observed in the lesions of alveolar, sheeted, and trabecular structures. Metastatic tumor cells originating from the epididymal mass were observed in the mesenteric, inguinal, and pancreatoduodenal lymph nodes. These metastatic lesions exhibited the same histopathological features as the left epididymal mass. The cauliflower-like mass in the scrotal dermis and forestomach mucosa was diagnosed as squamous cell papilloma. The swollen liver, spleen, and lymph nodes (submandibular, axillary, parathymus, hilus, renal, iliac, and popliteal) had been infiltrated by lymphoma cells. The results of immunohistochemical staining are summarized in Table 1. Epididymal tumor cells were positive for CK AE1/AE3 and CK14 (Fig. 4A and 4B, respectively), and a small number of tumor cells were Ki67-positive (Fig. 4C). The tumor cells were CK5, p63, uroplakin III (Fig. 4D), vimentin, desmin, and α-SMA negative.

Based on these histological findings, the mass was diagnosed as squamous cell carcinoma.

Squamous cell carcinoma of the epidermis and forestomach is classified into three subtypes: ‘well differentiated’, ‘moderately differentiated’, and ‘poorly differentiated’⁴,⁵. The present case lacked cancer pearls and distinct keratinization, and spindle-shaped tumor cells were predominant. These findings suggested moderate to poor differentiation. CK5 and p63 are known to be markers for classic squamous cell carcinoma⁶; however, the present case was negative for both markers. These results reflected poorly differentiated tumor cells. Transitional epithelial tumor was negated.

![Fig. 1. Macrosopic appearance of left epididymis. (a) Caput, white-grayish mass. (b) Corpus. (c) Cauda. (d) Adipose tissue.](image)

![Fig. 2. (A) The mass composed of nodules (arrows) and an infiltrative growth lesion (arrow heads). Hematoxylin-eosin (HE) stain. Bar=400 μm. (B) The nodules surrounded by thick fibrous connective tissue (arrows). Masson’s trichrome stain. Bar=400 μm.](image)
Fig. 3. (A) Polygonal tumor cells proliferated in an alveolar structure. Tumor cells exhibiting keratinization (*). HE stain. Bar=50 μm. (B) Alveolar structures characterized by argyrophilic fibers surrounding them. Periodic acid-methenamine-silver stain. Bar=50 μm. (C) Polygonal tumor cells proliferated in a sheeted structure. Tumor cells having formed intercellular bridges (arrows) and exhibiting cobblestone-like arrangement. HE stain. Bar=50 μm. Inset: intercellular bridges (arrows). HE stain. Bar=20 μm. (D) Irregularly shaped cavity formed in the alveolar structure (*). The cavity containing keratin-like material (arrow) and keratinocytes (arrow heads). HE stain. Bar=50 μm. (E) Nodular lesion. Epididymal ducts remaining in the nodular lesion (arrows). The ducts with preserved normal ductal structure. HE stain. Bar=100 μm. (F) Infiltrative growth lesion. Tumor cells: spindle-shaped (arrows) and proliferated in trabecular structure. HE stain. Bar=50 μm.
### Table 1. Results of Immunohistochemical Staining

| Antibody     | Positive cells in normal tissue | Tumor cells | Ductus epididymis (epithelial cells)* | Ductus epididymis (basal cells)* | Stromal cells |
|--------------|--------------------------------|-------------|--------------------------------------|----------------------------------|---------------|
|              |                                | Alveolar, sheeted structure (polygonal cells) | Trabecular structure (spindle-shaped cells) |                                  |               |
| Cytokeratin AE1/AE3 | Epithelial cell                 | +           | +                                    | +                                | −             |
| Cytokeratin 14 | Squamous and basal cell         | +           | +                                    | −                                | +             |
| Cytokeratin 5 | Keratinized epithelial cell     | −           | −                                    | −                                | −             |
| Uroplakin III | Transitional epithelial cell    | −           | −                                    | −                                | −             |
| p63          | Squamous and basal cell         | −           | −                                    | −                                | −             |
| Vimentin     | Mesenchymal cell                | −           | −                                    | −                                | +             |
| Desmin       | Muscle                          | −           | −                                    | −                                | −             |
| α-SMA        | Smooth muscle and myoepithelia  | −           | −                                    | −                                | +             |
| Ki 67        | Proliferative cell              | **         | **                                   | −                                | +**           |

+: positive, −: negative. *: in the mass, **: partly, a few cells.

**Fig. 4.** Immunohistochemical staining of tumor cells.
Tumor cells were positive for cytokeratin (CK) AE1/AE3 (A), CK 14 (B) and Ki67 (C). Tumor cells were negative for uroplakin III (D). Bars=100 μm (A, B, C). Bar=50 μm (D). Basal cells of remnant ductus epididymis were positive for CK14 (B, arrows).
by the result of uroplakin III, and the other tumors such as seminoma, Leydig cell tumor, rate testes tumor, and mesothelioma were negated by the histopathological features and immunohistochemical results.

A detailed histological observation of all organs revealed no squamous cell carcinoma in organs and/or tissues other than the left epididymis and the lymph nodes where metastases were found. Therefore, the squamous cell carcinoma was concluded to have originated in the left caput epididymis. The tumor cells were characterized by being CK14-positive. In the mouse epididymis, CK5 and CK14 have been reported to be specific markers of basal cells in the epididymal ducts. The basal cells of the remaining epididymal ducts in the mass were also positive for CK14, suggesting that the tumor cells derived from the basal cells of the epididymal ducts. In 1909, Rowlands et al. reported that squamous cell carcinoma of the human epididymis originates from the basal cells or epithelium of the ductus epididymis and that squamous cell carcinoma develops after inflammatory stimuli caused by squamous cell metaplasia. While squamous cell metaplasia in the epithelium of the ductus epididymis has been reported to occur in mice, Squamous cell metaplasia was not observed in the remaining epididymal duct in the present case. The tumor cells, in this case, originated from basal cells of the epididymal duct, but the pathogenesis from basal cells to tumor cells could not be clarified. It has been reported that rasH2-Tg mice develop tumors due to point mutations in the c-Ha-ras gene by various genotoxic agents, including MNU. Point mutations have also been reported in squamous cell papilloma arising in the forestomach and epidermis. Therefore, squamous cell papilloma of the scrotal dermis and the forestomach in the present case was considered to be induced by MNU. We did not investigate genetic mutation in the tumor tissue and could not prove a relationship between squamous cell carcinoma and MNU. In addition, there have been no reports of squamous cell carcinoma arising spontaneously in the epididymis of mice, and we could not conclude that this was a spontaneous lesion. Therefore, we could not conclude whether the present case was spontaneous or related to MNU administration or the c-Ha-ras gene.

To the authors’ knowledge, this case of primary squamous cell carcinoma of the epididymis is the first such report in a rasH2-Tg mouse.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest.

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