

Case Report

Spontaneous Basal Cell Carcinoma of the Submandibular Gland in a Rat

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Abstract: At necropsy, a white nodule (about 5 × 3 mm in size) was observed in the right submandibular gland of a 10-week-old female GALAS rat. Histopathologically, oval to spindle-shaped and pale basophilic tumor cells proliferated closely, and formed variably sized foci. The nodule partially spread into or invaded the surrounding normal tissue, and necrotic foci were recognized in the tumor. Immunohistochemically, the nuclei of the tumor cells showed a diffusely positive reaction for p63, and the cytoplasm showed a diffusely positive reaction for cytokeratin and negative reaction for α SMA, vimentin, desmin and S-100. Many tumor cells were positive for PCNA. Ultrastructurally, the tumor cells contained many tonofilaments in the cytoplasm and a few desmosomes at the intercellular portion. Based on these findings, the tumor was diagnosed as a basal cell carcinoma originating from the duct in the rat submandibular gland. (*J Toxicol Pathol* 2010; 23: 147–149)

Key words: basal cell, carcinoma, submandibular gland, rat

Introduction

Spontaneous tumors of the rat submandibular gland are very rare^{1,2}, and most of them are diagnosed as adenomas or adenocarcinomas originating from acinar cells and ductal epithelia³. To our knowledge, there are few reports on detailed analyses of tumors in the submandibular glands of rats.

In the present case, the results of immunohistochemical and ultrastructural observations indicated that the rat submandibular gland tumor was probably derived from basal cells located in the duct. The animal was a 10-week-old female GALAS rat (BrlHan:WIST@jcl, purchased from CLEA Japan, Inc., Tokyo, Japan) that was assigned to the control group in a 4-week repeated-dose oral toxicity study and subjected to the final sacrifice according to the experimental protocol. During the treatment period, there was no abnormality in clinical signs or body weight of the animal. At necropsy, a white nodule (about 5 × 3 mm in size) was found in the right submandibular gland. In other organs, there was no abnormality related to this tumor. The animal was treated in accordance with the Rules for Approval of Animal Experiments in Sugi Institute of Biological Science

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The submandibular gland was routinely fixed in 10% phosphate-buffered formalin, and embedded in paraffin and sectioned. Paraffin sections were stained with hematoxylin and eosin (HE). Immunohistochemical staining was performed using the following primary antibodies: cytokeratin (AE1/AE3; 1:20, DAKO, Denmark), p63 (1:50, DAKO, Denmark), alpha-smooth muscle actin (α SMA) (1:50, DAKO, Denmark), vimentin (1:50, DAKO, Denmark), desmin (1:100, DAKO, Denmark), S-100 (1:400, LVC, USA) and proliferating cell nuclear antigen (PCNA; 1:100, DAKO, Denmark). The deparaffinized sections were incubated in the antibodies at room temperature for 1 hr and then in the EnVison+System-HRP Labeled Polymer (DAKO, Denmark) at room temperature for 30 min, and the staining was visualized with diaminobenzidine. For transmission electron microscopic examinations, the tissue kept in 10% phosphate-buffered formalin was postfixed in 1% osmium, routinely processed to epoxy resin-embedded ultrathin sections and stained with uranyl acetate and lead citrate.

Histopathologically, oval to spindle-shaped and pale basophilic tumor cells proliferated closely and formed variably sized foci or grew diffusely in the submandibular gland (Figs. 1A, 1B). The nodule separated by thin interstitial connective tissue and lobular structures were partially formed (Fig. 1A). The border between the nodule and surrounding normal tissue was mainly clear, and the nodule partially spread into or invaded the surrounding normal tissue. Necrotic foci were recognized in the nodule (Fig. 1C). The

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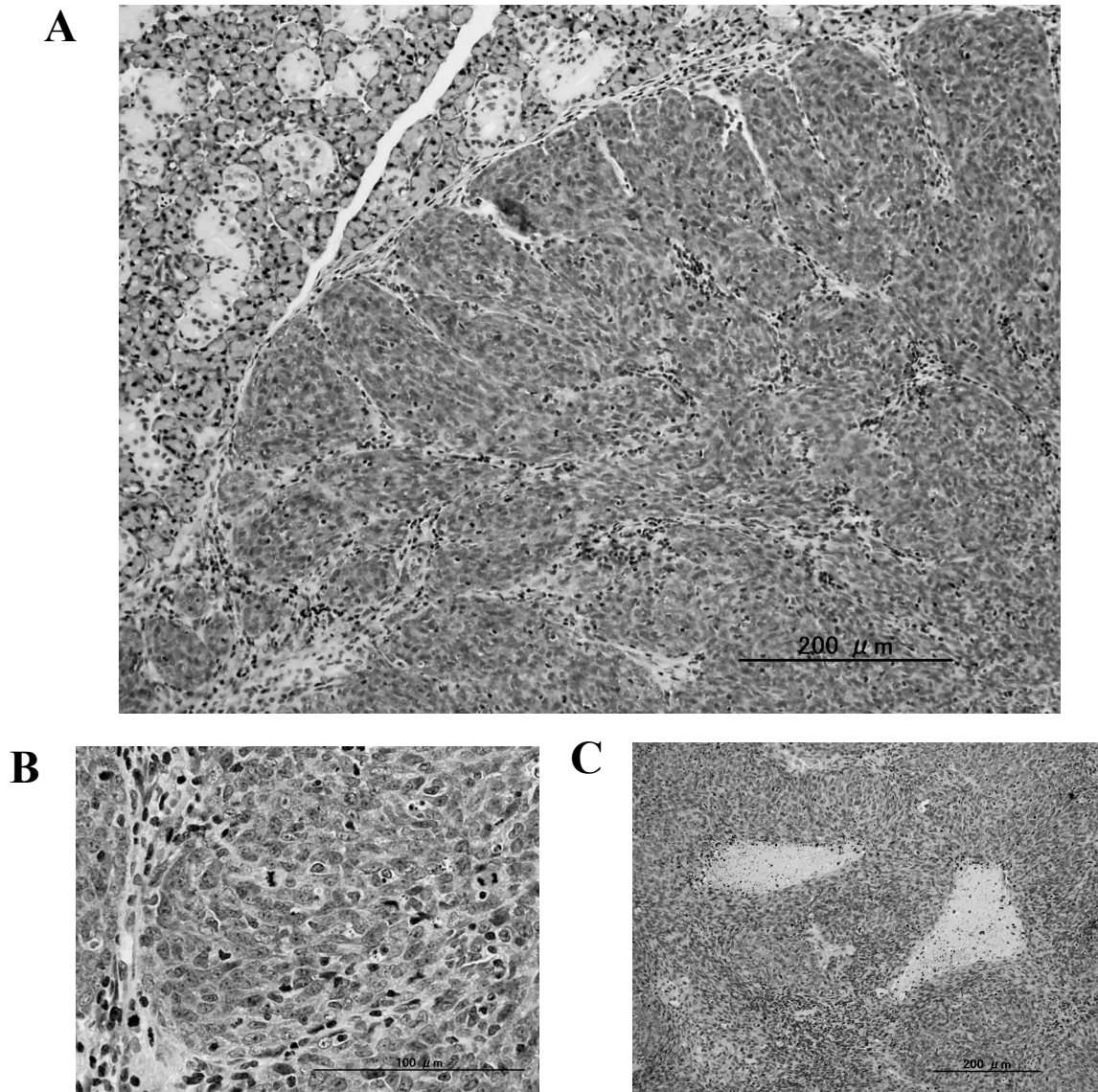


Fig. 1. The nodule in the submandibular gland. H-E stain. A: The nodule was separated by thin connective tissue, and lobular structures were partially formed. B: In the nodule, oval to spindle-shaped and pale basophilic tumor cells proliferated in close proximity. The nuclei of the tumor cells showed various sizes and many mitotic figures and contained poor chromatin and one to a few nucleoli. C: Necrotic foci were recognized in the nodule.

nuclei of the tumor cells showed various sizes and many mitotic figures and contained poor chromatin and one to a few nucleoli (Fig. 1B). Ductal and acinar structures, cribriform patterns and keratinization were not found in the tumor. A palisade arrangement of proliferating cells was not visible. Lymphocytic infiltration was slightly recognized in the thin connective tissue.

Immunohistochemically, the tumor cells showed a diffusely positive reaction for p63 in the nuclei (Fig. 2) and for cytokeratin (AE1/AE3) in the cytoplasm. There was a negative reaction for α SMA, vimentin, desmin and S-100. Many cells tested positive for PCNA in the tumor.

Ultrastructurally, the tumor cells contained many tonofilaments in their cytoplasm and a few desmosomes at

the intercellular portion (Fig. 3). The existence of tonofilaments revealed that these cells were epithelial cells. Myofilament was not recognized in the cytoplasm of the tumor cells.

The anti-p63 protein is a selective marker for both basal and myoepithelial cells^{4,5}, and the anti- α SMA protein is a selective marker for myoepithelial cells. In addition, the reliable identification method of myoepithelial cells is thought to be an immunohistochemical method using anti- α SMA protein, together with anti-vimentin protein or anti-S-100 protein^{6,7}. In the present case, the nuclei of the tumor cells were positive for anti-p63 protein, whereas no tumor cells showed a positive reaction for anti- α SMA protein. Furthermore, the tumor cells were positive for cytokeratin

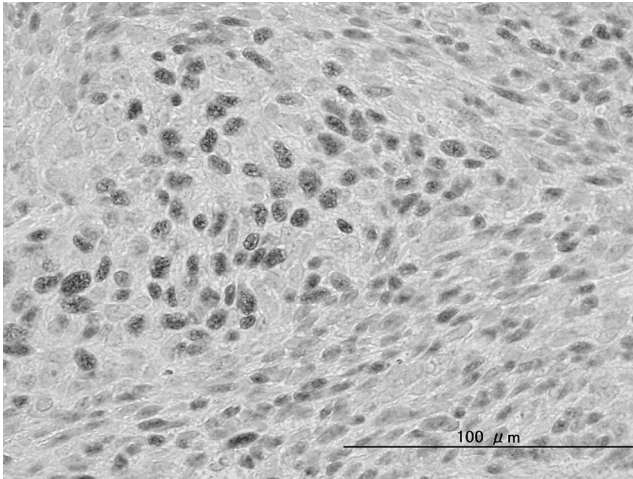


Fig. 2. Immunohistochemically, the nucleus of the tumor cell showed a diffusely positive reaction for p63.

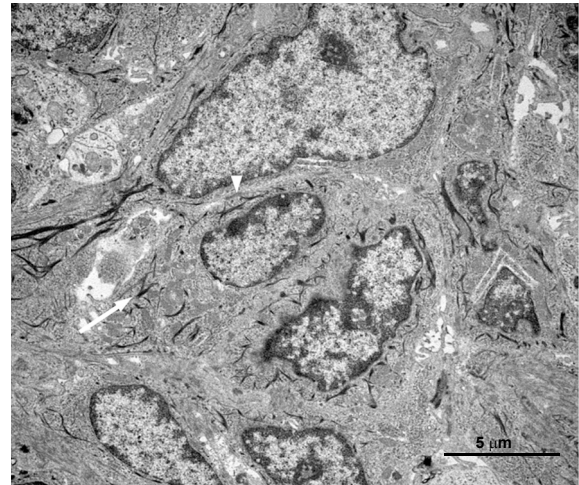


Fig. 3. Ultrastructurally, the tumor cell contained many tonofilaments (arrow) in the cytoplasm and a few desmosomes (arrow heads) at the intercellular portion.

(AE1/AE3) and negative for vimentin, S-100 and desmin. Based on the results of immunohistochemical examinations, the tumor cells were considered to be derived from basal cells.

Ultrastructurally, myoepithelial cells generally possess myofilaments with dense bodies⁸. In the present case, the tumor was not considered to originate from myoepithelial cells because there were no myofilaments in the cytoplasm. This ultrastructural finding supported the immunohistochemical results.

Since the tumor cells had many mitotic figures, a positive reaction to anti-PCNA protein, and partially necrotic foci and infiltrated into the surrounding normal tissue, the tumor was regarded as a malignant tumor.

Based on these results, the tumor was diagnosed as a spontaneous basal cell carcinoma. In the normal submandibular gland, basal cells are located in the excretory duct³; however, tonofilaments, the existence of which is one of the characteristic features of squamous cells, were observed in the cytoplasm of the tumor cells. Squamous cells are normally located in the end portion of the excretory duct in the submandibular gland^{3,9}. Therefore, the tumor is considered to have originated from the basal cells in the excretory duct near the opening to the oral cavity. We did not use the diagnostic term ‘adenocarcinoma’ because this tumor did not clearly show the ductal structure.

In humans, tumors of the submandibular gland originating from basal cells are classified as ‘basal cell adenomas’ or ‘basal cell adenocarcinomas.’ The histologic appearance of the basal cell adenoma is dominated by relatively uniform, monomorphous proliferation of basaloid cells. While each tumor generally has a uniform histomorphologic architecture, variations among tumors allow categorization into solid, trabecular, tubular and membranous types. Basal cell adenocarcinoma is very similar to basal cell adenoma cytologically and histomorphologically and manifests infiltrative

growth and incidental metastasis⁹. In rats, however, there are no reports on the classification of basal cell adenomas or adenocarcinomas in the submandibular gland.

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