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Case Report

Undifferentiated Sarcoma of the Salivary Gland in a Mongolian Gerbil (*Meriones unguiculatus*)

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Abstract: A subcutaneous mass was found in the lower ventral neck region of a 55-week-old male Mongolian gerbil (*Meriones unguiculatus*). Histopathologically, the mass involved salivary glands and featured diffuse proliferation of pleomorphic neoplastic cells with large necrotic foci. The lesion was well demarcated from the surrounding tissue, although invasive growth to fibrous septa was occasionally observed. The neoplastic cells were mainly arranged in irregular sheets with severe cellular atypia, round to oval nuclei and varying amounts of eosinophilic cytoplasm. Mitotic figures and multinucleated giant cells were frequent. Immunohistochemical analysis revealed that the neoplastic cells were strongly positive for vimentin and S-100 and negative for NSE, cytokeratin, α-SMA, c-kit, factor VIII, CD34, α-1-antitrypsin, lysozyme and MSR-A. Based on the results, the mass was diagnosed as an undifferentiated sarcoma of the salivary gland. To the best of our knowledge, this is the first report of such a tumor in Mongolian gerbils. (DOI: 10.1293/tox.24.173; J Toxicol Pathol 2011; 24: 173–177)

Key words: undifferentiated sarcoma, salivary gland, Mongolian gerbil

Primary salivary gland tumors of rodents occur infrequently[1,2], most being diagnosed as adenomas or adenocarcinomas derived from acinar cells and ductal epithelium. Fibrosarcoma, rhabdomyosarcoma, hemangiosarcoma, schwannoma and undifferentiated sarcoma are all known as mesenchymal neoplasms originating in the salivary glands of rodents but are much more rare.

Mongolian gerbils (*Meriones unguiculatus*) have been widely used as experimental rodent models[3–5], and there have been several reports of spontaneous tumors in aging animals such as sebaceous gland carcinoma of the skin, granulosa cell tumor of the ovary and cortical adenoma of the adrenal gland[6–8]. However, there have been no descriptions of salivary gland tumors, spontaneous or chemically induced, in gerbils. In this article, we report a case of an undifferentiated sarcoma of the salivary gland in a male at 55 weeks of age.

The case was found in a group of male Mongolian gerbils (MGS/Sea, Kyudo Co., Ltd., Saga, Japan), employed as positive control animals in a gastric carcinogenesis study. The gerbil was infected with *Helicobacter pylori* (*H. pylori*) by oral gavage at 6 weeks of age and received administration of 10 ppm *N*-methyl-*N*-nitrosourea (MNU) in drinking water from 8 to 28 weeks of age. It was housed in a plastic cage on hardwood chip bedding in an air-conditioned biohazard room with a 12-h light/12-h dark cycle and allowed free access to CE-2 powder diet (CLEA Japan, Tokyo, Japan) and tap water throughout the experiment. The experimental design was approved by the animal care committee of Aichi Cancer Center Research Institute, and all animals were cared for in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1st, 2006). The animal was sacrificed under deep anesthesia at 55 weeks of age because of weakness caused by

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Table 1. Antibodies Used for Immunohistochemistry

Antigen	Clonality (clone)	Host species	Dilution	Antigen retrieval	Supplier	Reactivity[1]
Vimentin	Mono (V9)	Mouse	1:100	Autoclave	DAKO, Glostrup, Denmark	+
S-100	Poly	Rabbit	Ready-to-use	None	DAKO	+
NSE	Mono (H14)	Mouse	Ready-to-use	None	DAKO	–
Cytokeratin (wide)	Poly	Rabbit	1:500	Protease	DAKO	–
Cytokeratin	Mono (AE1/AE3)	Mouse	1:50	Protease	DAKO	–
Desmin	Mono (D33)	Mouse	Ready-to-use	None	DAKO	±
Sarcomeric actin	Mono (alpha-Sr-1)	Mouse	1:50	Autoclave	DAKO	±
Alpha-SMA	Mono (1A4)	Mouse	1:50	Autoclave	DAKO	–
c-Kit (CD117)	Poly	Rabbit	1:300	Autoclave	DAKO	–
Factor VIII	Poly	Rabbit	1:200	Autoclave	DAKO	–
CD34	Mono (QBEnd-10)	Mouse	1:50	Protease	DAKO	–
Alpha-1-antitrypsin	Poly	Rabbit	Ready-to-use	Protease	DAKO	–
Lysozyme	Poly	Rabbit	Ready-to-use	Protease	DAKO	–
MSR-A	Mono (SRA-E5)	Mouse	1:50	Microwave	TransGenic Inc., Kobe, Japan	–

[1] –, negative; ±, scattered positive; +, diffusely positive. NSE, neuron-specific enolase; SMA, smooth muscle actin; Factor VIII, Von Willebrand factor; MSR-A, macrophage scavenger receptor-A.

feeding difficulties. At necropsy, a large subcutaneous mass involving the salivary gland was identified in the lower ventral neck region. The lesion was 3.7 × 3.3 × 3.0 cm in size, covered with thin connective tissue and well demarcated from surrounding tissues. The cut surface was red-brown in color with extensive necrotizing hemorrhage. After removal and fixation in 10% neutral-buffered formalin for 24 hours, slices were routinely embedded in paraffin, and four-μm thick sections were prepared and stained with hematoxylin and eosin (H&E) for histological observation. Immunohistochemical staining for vimentin, S-100, neuron-specific enolase (NSE), cytokeratin (keratin-wide and AE1/AE3), desmin, sarcomeric actin, α-smooth muscle actin (α-SMA), c-kit (CD117), Von Willebrand factor (factor VIII), CD34, α-1-antitrypsin, lysozyme and macrophage scavenger receptor A (MSR-A) was also performed using serial sections. Table 1 provides details of the sources of the antibodies and conditions for the immunohistochemistry.

Histopathologically, the mass consisted of a diffuse proliferation of neoplastic cells with significant atypia and massive necrosis with hemorrhage (Fig. 1a). Demarcation by thin connective tissue was noted, although invasive growth to fibrous septa was occasionally observed. The neoplastic cells were mainly arranged in an irregular sheet pattern or occasionally in epithelial-like clusters with unclear cell borders. The cells were highly pleomorphic and had scant to abundant eosinophilic cytoplasm, sometimes with vacuolar degeneration. The nuclei of neoplastic cells were round to oval in shape and occasionally contained a few prominent eosinophilic nucleoli (Fig. 1b). Multinuclear giant cells were often observed, and mitotic figures were also abundant. Residual atrophic ducts were scattered in the tumor, especially near the adjacent salivary gland tissue (Fig. 1c). In the other organs, there were no histopathological findings except for severe chronic gastritis associated with *H. pylori* infection. No similar lesions of the salivary gland were observed in any of the other gerbils used in the experiment.

On immunohistochemical analysis, the neoplastic cells were strongly positive for vimentin (Fig. 2a) and S-100 (Fig. 2b) in the cytoplasm and negative for cytokeratin (keratin-wide and AE1/AE3; Figs. 2c and d). A few tumor cells showed positive staining for desmin (Fig. 2e) and sarcomeric actin (Fig. 2f), but most were negative. The neoplastic cells were also negative for NSE, α-SMA (Fig. 2g), c-kit (Fig. 2h), factor VIII (Fig. 2i), CD34 (Fig. 2j), α-1-antitrypsin (Fig. 2k), lysozyme (Fig. 2l) and MSR-A (Fig. 2m). The results of immunohistochemistry are summarized in Table 1.

The histopathological observations suggested that the mass of the present case was derived from the salivary gland tissue. Based on the morphological findings, poorly-differentiated adenocarcinoma, myoepithelioma, schwannoma, leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, hemangiosarcoma, histiocytic sarcoma and undifferentiated sarcoma were considered for the differential diagnosis. The results of immunostaining for vimentin and cytokeratin revealed that the tumor cells were of mesenchymal rather than epithelial origin. Myoepithelial carcinomas of the salivary gland in humans have been reported to be consistently positive for cytokeratin (AE1/AE3)[9] and occasionally positive for c-kit[10]. In addition, Sundberg et al. demonstrated that murine myoepitheliomas in the salivary gland were unequivocally positive for keratin 5 and 14, both of which should be recognized by AE1/AE3[11]. Thus, the tumor was unlikely to be of a myoepithelial origin. NSE, α-SMA and factor VIII are known to be typical markers for tumors originating from neuroendocrine cells, smooth muscle and the vascular endothelium, respectively. In addition, CD34, α-1-antitrypsin, lysozyme and MSR-A are used as histiocytic markers. Therefore, the negative reactions with their antibodies in the present case do not support any possibility of neuroendocrine tumor, leiomyosarcoma, hemangiosarcoma and histiocytic sarcoma. Schwannomas and fibrosarcomas are generally characterized by bundle formation of spindle cells. However, most neoplastic cells in the pres-

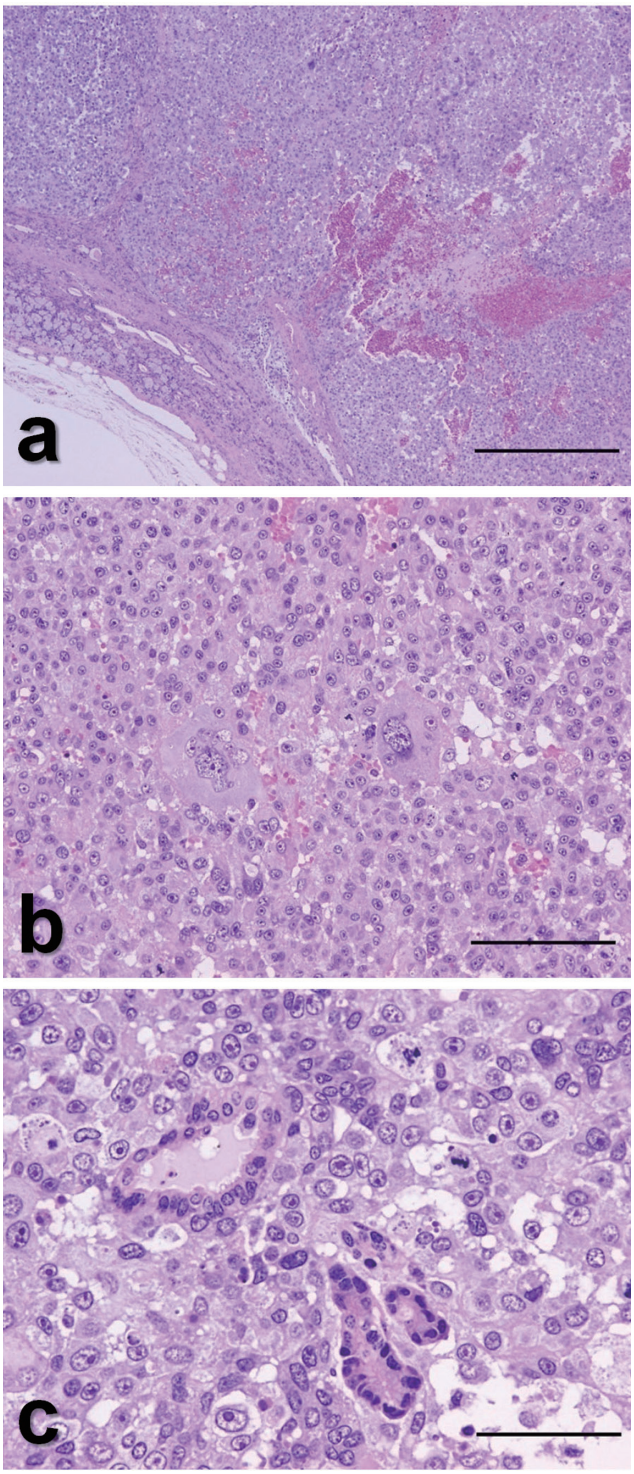

Fig. 1. Histopathological findings for the subcutaneous mass located in the lower ventral neck region of the Mongolian gerbil. (a) The mass adjacent to the parotid gland (lower left) consists of diffusely proliferating neoplastic cells often with hemorrhage and necrosis (H&E; bar = 500 μm). (b) Neoplastic cells show severe pleomorphism and atypia, and multinuclear giant cells are frequent (H&E; bar = 100 μm). (c) Note atrophic ducts of salivary glands remaining in the neoplastic tissue (H&E; bar = 50 μm).

ent gerbil had round to polygonal cytoplasm and were arranged in irregular sheets, sometimes with epithelial-like nests. Although the expression of desmin and sarcomeric actin suggested a myogenic origin, the positive cells were only scattered in limited regions of neoplastic tissue. Taken together, there was no evidence to indicate any specific differentiation of the neoplasm in the present case. Therefore, the mass was diagnosed as an undifferentiated sarcoma of the salivary gland.

In rats, naturally-occurring or chemically-inducible mesenchymal tumors in the salivary glands are classified into at least four types, including undifferentiated sarcoma, malignant schwannoma, fibrosarcoma and rhabdomyosarcoma[1]. The term undifferentiated sarcoma is used for diagnosis of poorly-differentiated mesenchymal tumors for which the cell of origin cannot be determined[1]. Histopathological findings of undifferentiated sarcoma are characterized by diffuse proliferation of pleomorphic epithelial-like to spindle-shaped neoplastic cells, with occasional multinucleated giant cells, invasive growth and remnants of atrophic ducts[1,12], which is consistent with the findings for the present case. In humans and other domestic animals, sarcomas of the salivary gland without specific differentiation are not well established as diagnostic criteria because they are extremely rare[13,14]. Therefore, it is considered that the term "undifferentiated sarcoma" defined in rats may be appropriate as the diagnosis for the present case.

In summary, we report here a case of malignant mesenchymal tumor located in the salivary gland of a male Mongolian gerbil. In line with the lack of specific differentiation of neoplastic cells in terms of morphology and immunohistochemical characteristics, the mass was diagnosed as an undifferentiated sarcoma of the salivary gland. The present case was detected in only one gerbil out of fifty MNU-treated animals that we examined. In addition, no salivary gland tumors have been reported with single doses of MNU in rodent models[15]. Therefore, this first reported salivary gland tumor in a Mongolian gerbil may be incidental and not be related to the carcinogen.

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References

1. Elwell MR, and Leininger JR. Tumours of the salivary and lacrimal glands. In: Pathology of Tumours in Laboratory Animals Volume 1 - Tumours of the Rat, 2nd ed., VS Turusov, and U Mohr (eds). International Agency for Research on Cancer, Lyon. 89–107. 1990.
2. Frith CH, and Heath JE. Tumours of the salivary gland. In: Pathology of Tumours in Laboratory Animals Volume II -

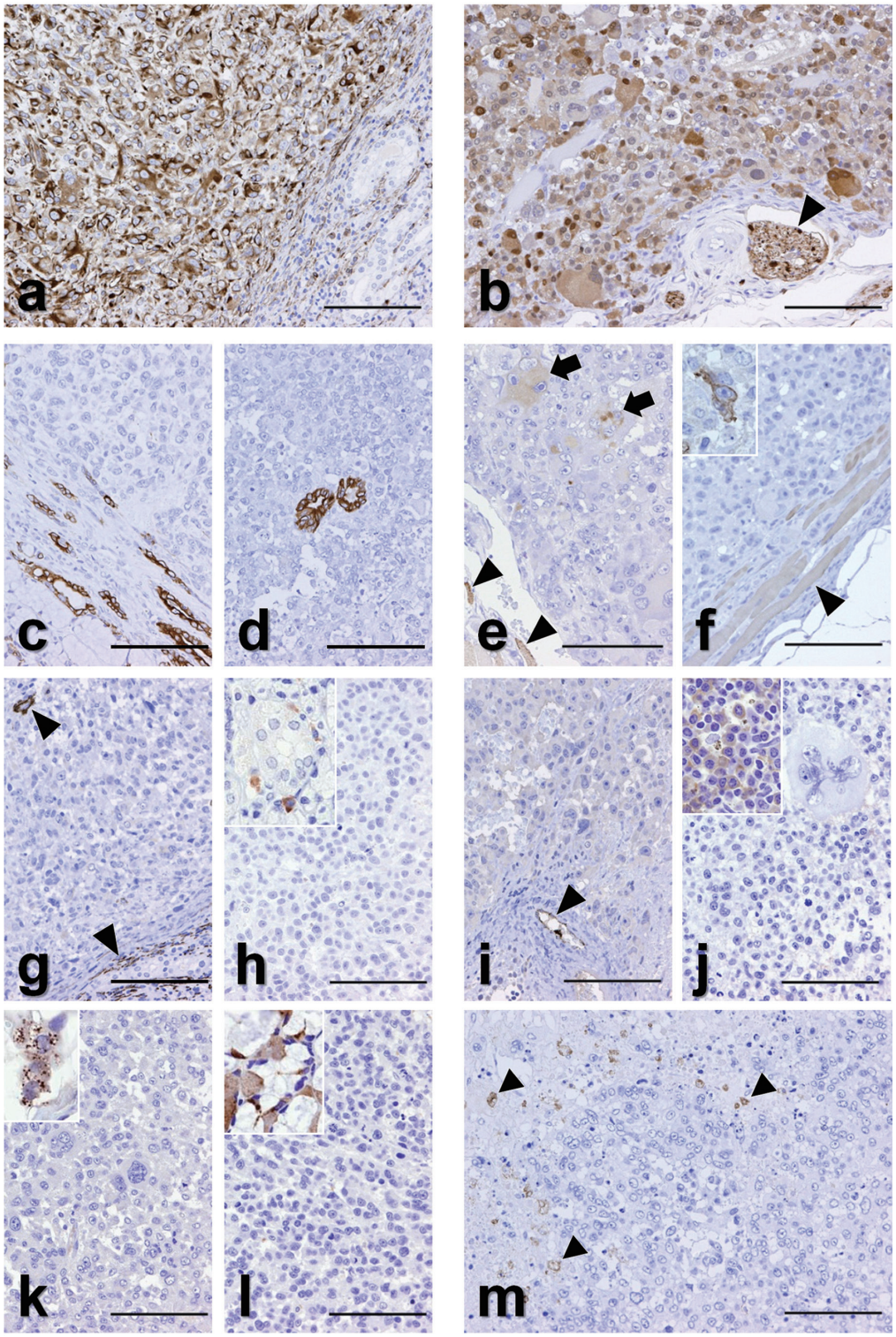

Fig. 2. Immunohistochemical findings for neoplastic tissue in the salivary gland of the Mongolian gerbil. Bars = 100 µm. (a) Vimentin. Neoplastic cells and the stromal tissue of the adjacent salivary gland are positive. (b) S-100. Neoplastic cells and peripheral nerve (arrowhead) are positive. (c and d) Cytokeratin (AE1/AE3). Epithelial cells in normal and residual salivary gland ducts are positive, while the neoplastic cells are negative. (e and f) Desmin and sarcomeric actin, respectively. Striated muscle tissue (arrowheads) and a few neoplastic cells (arrows in e and insert in f) are positive. (g) α-SMA. Vascular smooth muscles (arrowheads) are positive, while the neoplastic cells are negative. (h) c-Kit. Myoepithelial cells in the adjacent salivary gland (insert) are positive, while the neoplastic cells are negative. (i) Factor VIII. The vascular endothelium (arrowhead) is positive, while the neoplastic cells are negative. (J) CD34. Histiocytes in the adjacent lymph node (insert) are positive, while the neoplastic cells are negative. (k) α-1-Antitrypsin. Macrophages (insert) are positive, while the neoplastic cells are negative. (l) Lysozyme. Acinar cells in the adjacent salivary gland (insert) are positive, while the neoplastic cells are negative. (m) MSR-A. Macrophages infiltrating a necrotic area (arrowheads) are positive, while the neoplastic cells are negative.

Tumours of the Mouse, 2nd ed., VS Turusov, and U Mohr (eds). International Agency for Research on Cancer, Lyon. 115–139. 1994.
3. Tsukamoto T, Mizoshita T, and Tatematsu M. Animal models of stomach carcinogenesis. Toxicol Pathol. **35**: 636–648. 2007. [Medline] [CrossRef]
4. Ikeda K, Negishi H, and Yamori Y. Antioxidant nutrients and hypoxia/ischemia brain injury in rodents. Toxicology. **189**: 55–61. 2003. [Medline] [CrossRef]
5. Tatematsu M, Tsukamoto T, and Mizoshita T. Significant factors on gastric carcinogenesis revealed by experimental animal models. J Toxicol Pathol. **19**: 75–86. 2006. [CrossRef]
6. Meckley PE, and Zwicker GM. Naturally-occurring neoplasms in the Mongolian gerbil, *Meriones unguiculatus*. Lab Anim. **13**: 203–206. 1979. [Medline] [CrossRef]
7. Guzmán-Silva MA, Rossi MI, and Guimaraes JS. Craniopharyngioma in the Mongolian gerbil (*Meriones unguiculatus*): a case report. Lab Anim. **22**: 365–368. 1988. [Medline] [CrossRef]
8. Vincent AL, Rodrick GE, and Sodeman WA, Jr. The pathology of the Mongolian Gerbil (*Meriones unguiculatus*): a review. Lab Anim Sci. **29**: 645–651. 1979. [Medline]
9. Savera AT, Sloman A, Huvos AG, and Klimstra DS. Myoepithelial carcinoma of the salivary glands: a clinicopathologic study of 25 patients. Am J Surg Pathol. **24**: 761–774. 2000. [Medline] [CrossRef]
10. Jeng YM, Lin CY, and Hsu HC. Expression of the c-kit protein is associated with certain subtypes of salivary gland carcinoma. Cancer Lett. **154**: 107–111. 2000. [Medline] [CrossRef]
11. Sundberg JP, Hanson CA, Roop DR, Brown KS, and Bedigian HG. Myoepitheliomas in inbred laboratory mice. Vet Pathol. **28**: 313–323. 1991. [Medline] [CrossRef]
12. Neuenschwander SB, and Elwell MR. Salivary glands. In: Pathology of the Fischer Rat. GA Boorman, SL Eustis, MR Elwell, CA Montgomery Jr, and WF MacKenzie (eds). Academic Press, San Diego. 31–42. 1990.
13. Gnepp DR. Tumours of the Salivary Glands; Soft tissue tumours. In: WHO Classification: Head and Neck Tumours. L Barnes, JW Eveson, P Reichart, and D Sidransky (eds). IARC Press, Lyon. 275. 2005.
14. Brown CC, Baker DC, and Barker IK. Alimentary system. In: Pathology of Domestic Animals 5th ed. Volume 2, MG Maxie (ed). Elsevier, Edinburgh. 2007.
15. Parkin R, and Neale S. The effect of isoprenaline on induction of tumours by methyl nitrosourea in the salivary and mammary glands of female Wistar rats. Br J Cancer. **34**: 437–443. 1976. [Medline] [CrossRef]