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Case Report

Spontaneous Extraskeletal Osteosarcoma in the Stomach of an Aged F344 Rat

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Abstract: Extraskeletal osteosarcoma is a very rare tumor in humans and animals including rats. This paper describes a case of extraskeletal osteosarcoma observed in the glandular stomach of an aged female Fischer 344 rat. Grossly, a whitish solid mass was observed at the greater curvature of the glandular stomach. Histologically, the tumor consisted of both atypical polygonal and pleomorphic spindle-shaped cells, with pleomorphic nuclei, and it contained variable amounts of osteoids and small clumps of mature bone tissue. In addition, mitotic figures were frequently observed. Neither invasion of the muscle layer or vessels in the stomach nor metastasis to distant organs was detected. There were no skeletal tumors in the body. Immunohistochemically, the tumor cells were positive for osteocalcin, osteonectin, vimentin and S-100 protein. Judging from these results, the present tumor was diagnosed as extraskeletal osteosarcoma. This is the first report of spontaneous extraskeletal osteosarcoma arising from the stomach in a rat. (J Toxicol Pathol 2010; **23**: 157–159)

Key words: extraskeletal osteosarcoma, stomach, spontaneous, rat

Extraskeletal osteosarcoma, which occurs in tissues having no relation to the bone or periosteum, is a very rare tumor in humans and animals, and there are only a few reports of its occurrence in rats. In rats, extraskeletal osteosarcoma has been found in the subcutaneous tissue[1], thoracic cavity[2] and cecum[3]. The present paper describes the first case of spontaneous extraskeletal osteosarcoma observed in the glandular stomach of a rat.

The animal was a female F344/DuCrj rat (Charles River Laboratories Japan Inc., Kanagawa, Japan) that served as a control in a 2-year carcinogenicity study and was sacrificed as scheduled after the 2-year experimental period. The animals were housed individually in suspended stainless-steel wire mesh cages in an animal room that was maintained with a room temperature of 23 ± 3°C, relative humidity of 50 ± 20% and with air ventilation 12 to 17 times per hour and 12-hour illumination (7:00 to 19:00). Pellet diet (irradiation sterilized CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were provided *ad libitum*. The experiment was conducted in compliance with the laws or guidelines relating to animal welfare, including the "Standards Relating to the Care and Management, etc. of Experimental Animals" (Notification No. 6 of the Prime Minister's Office, Japan, March 27, 1980) and "Guidelines for Animal Experimentation" (the Japanese Association for Laboratory Animal Science, May 22, 1987).

Clinically, there were no distinct abnormal signs observed except for a body weight decrease from 285 g to 265 g with slight anorexia during the last week.

Macroscopically, a whitish solid mass that was 18 × 18 × 18 mm in size was observed at the greater curvature of the glandular stomach (Fig. 1). The mass protruded into the gastric cavity, and its surface was concave. There were no macroscopical findings indicating skeletal tumors in the body. After complete necropsy, all tissues including the tumor mass were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). Additional sections from the tumor at an almost equal ratio were stained with Masson's trichrome and were also subjected to immunohistochemistry by the peroxidase-labeled polymer method using an EnVision kit (DAKO, Glostrup, Denmark). The primary antibodies used were osteocalcin (polyclonal, 1:100, DAKO), osteonectin (polyclonal, 1:100, DAKO), vimentin (monoclonal, V9, 1:100, DAKO), S-100 (polyclonal, 1:500, DAKO), α-smooth muscle actin (α-SMA, monoclonal, 1A4, 1:100, DAKO) and keratin (polyclonal, 1:500, DAKO).

Histologically, the tumor was located mainly in the lamina propria mucosae of the glandular stomach, partially involved an upper part of the submucosa through the lamina muscularis mucosae and partially pressed the normal glands to the periphery. The tumor consisted of both polygonal

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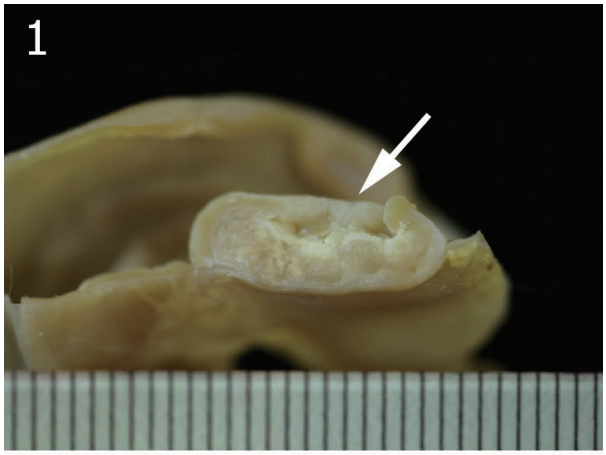
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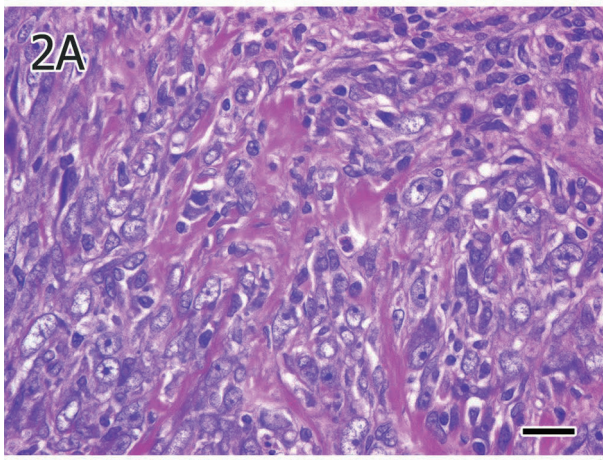
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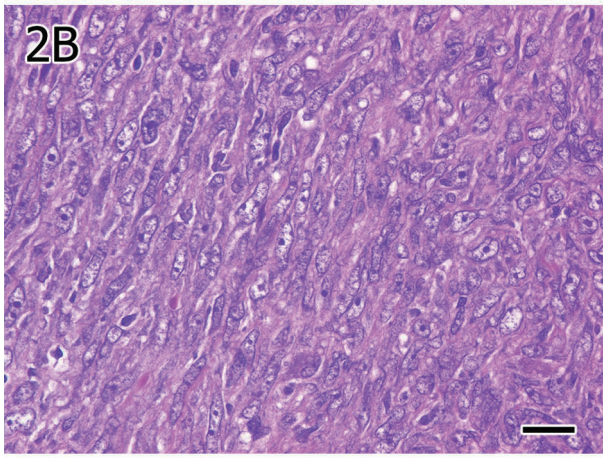
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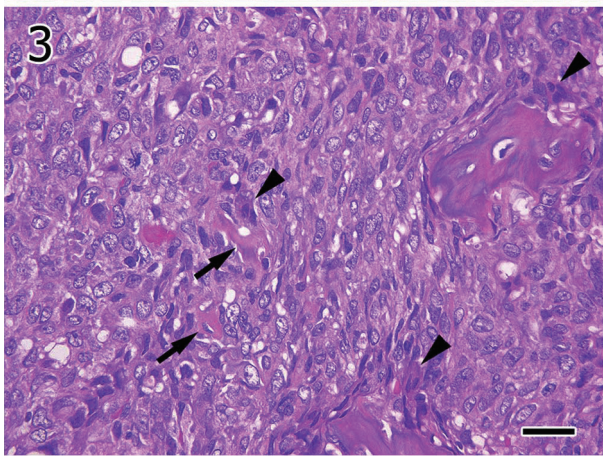
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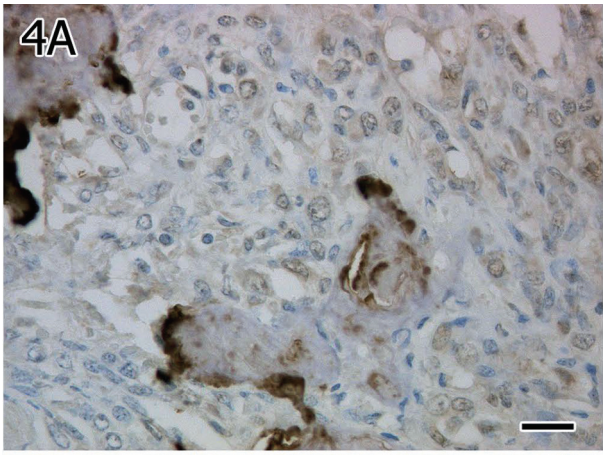
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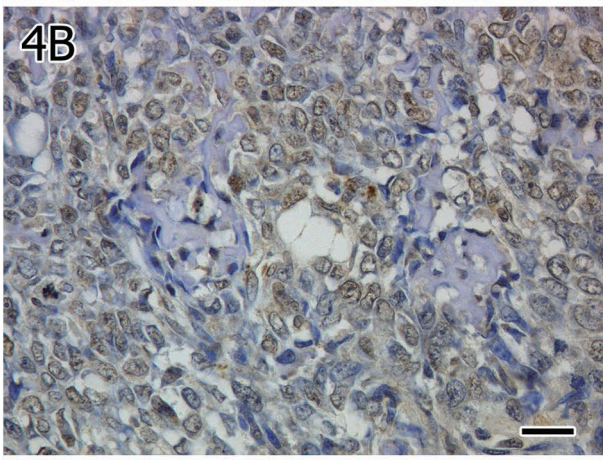
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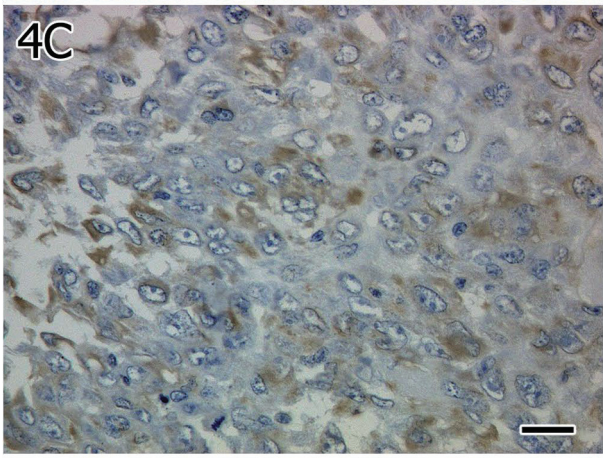
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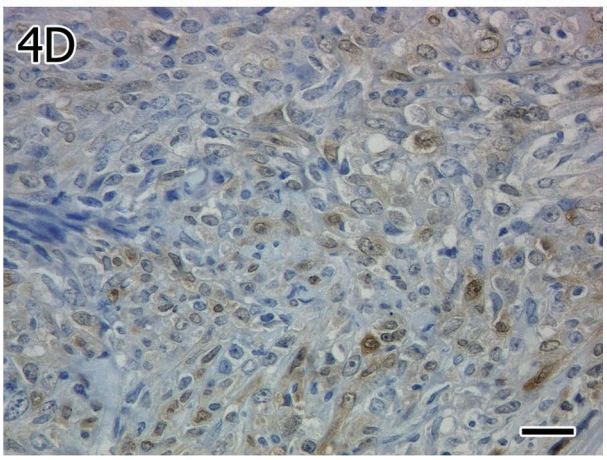
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cells suggesting atypical osteoblasts and spindle-shaped cells, and both neoplastic cells generally had a scantly basophilic or faintly eosinophilic cytoplasm and a pleomorphic plump nucleus with one or two prominent nucleoli (Fig. 2). The polygonal cells were closely packed together and were occasionally surrounded by lace-like osteoids (Fig. 2). A small clump of mature bone tissue was sporadically formed in the tumor, and a few multinucleated cells resembling osteoclasts were detected in the area of polygonal cell proliferation (Fig. 3). In addition, mitotic figures were frequently observed. Neither invasion of the muscle layer or vessels in the stomach nor metastasis to distant organs was observed. Masson's trichrome staining revealed that fibrous tissues were scarcely observed in the matrix. Immunohistochemically, both polygonal and spindle shaped neoplastic cells were positive for anti-osteocalcin, osteonectin, vimentin and S-100 protein (Fig. 4), but negative for anti-keratin and α-SMA. Immunoreactivity for osteocalcin, osteonectin and S-100 protein was noted in both the cytoplasm and nucleus of the neoplastic cells, while immunoreactivity for vimentin was found only in the cytoplasm. Moreover, strong immunoreactivity for osteocalcin was also found in the marginal bone matrix. Osteocalcin and osteonectin are reliable makers for osteogenic tumors, and some osteosarcomas positively react with anti-vimentin, S-100 protein and α-SMA antibodies[1,4]. There were no morphological or immunohistochemical evidence that neoplastic epithelial elements were involved in the tumor. Judging from these results, the present tumor was diagnosed as an extraskeletal osteosarcoma originating from the glandular stomach.

In addition to the above-mentioned tumor, the animal had various spontaneous and age-related lesions including anterior adenoma in the pituitary, endometrial stromal polyp in the uterus, large granular lymphocytic leukemia, adenoma in the adrenal, chronic progressive nephropathy in the kidney, retinal atrophy in the eye and rodent progressive cardiomyopathy.

Rat osteosarcoma is classified into the osteoplastic, fibroblastic, osteoblastic, telangiectatic and compound types according to the major components of the tumors[5] as follows: 1) the osteoplastic type is composed mostly of osteoids with scattered areas in which osteoblasts predominate; 2) the fibroblastic type is composed of spindle-shaped cells and a variable amount of osteoids; 3) the osteoblastic type is similar to the fibroblastic type, but the cells are polygonal or rounded; 4) the telangiectatic type is composed of blood-filled cystic sinuses with variable amounts of osteoids and osteoblasts in the intervening tissue, and multinucleated osteoclast-like cells may be numerous; and 5) the compound type is composed of both cartilaginous and osteoid tumor tissues. As mentioned above, the neoplastic cells of the present extraskeletal osteosarcoma in the stomach of a rat were composed of both osteoblast-like polygonal cells and spindle-shaped cells at an almost equal ratio. Taking into consideration that there were many neoplastic cells showing an apparent differentiation to osteoblasts, the present extraskeletal osteosarcoma may be subclassified as the osteoblastic type.

In conclusion, this paper reported the first case of spontaneous gastric extraskeletal osteosarcoma in a rat.

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Fig. 1. Macroscopic appearance of the stomach. The mass (arrow) formed at the greater curvature of the glandular stomach and protruded into the gastric cavity with a concave surface.

Fig. 2. Neoplastic cells. A) Polygonal cells surrounded by lace-like osteoids, and B) spindle-shaped cells. HE stain. Bar=20 μm.

Fig. 3. Osteoids (arrows) and mature bone formation with multinucleated cells (arrowheads). HE stain. Bar=20 μm.

Fig. 4. Immunohistochemistry. Positive reaction to A) osteocalcin in the cytoplasm and nucleus of neoplastic cells and marginal bone matrix, B) osteonectin in the cytoplasm and nucleus of neoplastic cells, C) vimentin in the cytoplasm of neoplastic cells and D) S-100 protein in the cytoplasm and nucleus of neoplastic cells. Bar=20 μm.