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Case Report

Spontaneous extraskeletal osteosarcoma in the duodenum of a Crlj:CD1 (ICR) mouse

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Abstract: Extraskeletal osteosarcoma is a very rare tumor in humans and animals. This paper describes a case of extraskeletal osteosarcoma observed in the duodenum of a male ICR mouse. Grossly, a solid mass pushing up the tunica serosa was observed in the duodenal wall. Histologically, the tumor was located in the lamina propria mucosae and tela mucosa. Neoplastic cells densely proliferated in these areas, and replaced of the normal tissue components. A small amount of osteoid and a small clump of bone tissue were observed in the area of neoplastic cell proliferation, especially in the lamina propria mucosae. Neoplastic cells consisted of atypical polygonal cells and pleomorphic spindle-shaped cells, and the former were predominant. Mitotic figures were occasionally observed. Neither invasion of vessels in the duodenum nor metastasis to distant organs was observed. There were no skeletal tumors in the body. Immunohistochemically, the neoplastic cells were positive for anti-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 duodenum of a mouse. (DOI: 10.1293/tox.2016-0043; J Toxicol Pathol 2016; 29: 275–278)

Key words: extraskeletal osteosarcoma, duodenum, spontaneous tumor, mouse

Extraskeletal osteosarcoma, which occurs in tissues having no relation to the bone or peritoneum, is a very rare tumor both in humans and animals, and there are only a few reports of its occurrence in rats and mice. Specifically, it has been reported in the skin (mouse)[1], subcutaneous tissue (rat)[2, 3], thoracic cavity (rat)[4], abdominal wall (mouse)[5], stomach (rat)[6], and cecum (rat)[7]. This paper describes the first case of spontaneous extraskeletal osteosarcoma detected in the duodenum of a mouse.

The animal was a male Crlj:CD-1 (ICR) mouse purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan) that served as a monitor in a 2-year carcinogenicity study and was found dead at 75 weeks of age. The animals used in the study were housed individually in suspended stainless-steel wire mesh cages in an animal room maintained at a temperature of 23 ± 3°C and relative humidity of 50 ± 20% with air ventilation 12 to 17 times per hour and 12 hours of 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 (Japanese Association for Laboratory Animal Science, May 22, 1987).

Macroscopically, a solid mass (6 × 5 × 5 mm in size) pushing up the tunica serosa was palpable from the serous surface of the duodenum (Fig. 1). The mass, which showed a whitish cut surface, was located in the duodenal wall and did not protrude into the duodenal cavity. There were no macroscopic findings indicating skeletal or other 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 mass were also subjected to immunohistochemistry by the peroxidase-labeled polymer method using an EnVision+ kit (anti-rabbit or anti-mouse, Dako, Glostrup, Denmark). The primary antibodies used were osteocalcin (polyclonal, 1:100, Santa Cruz Biotechnology, Dallas, TX, USA), osteonectin (polyclonal, 1:500, LSL, Tokyo, Japan), vimentin (polyclonal, 1:100, Abcam, Cambridge, UK), S-100 (polyclonal, 1:500, Dako), Iba-1 (polyclonal, 1:100, Wako Pure Chemical Industries, Osaka, Japan), α-smooth muscle actin (α-SMA) (monoclonal, 1:100, Dako), and keratin/cytokeratin (polyclonal, ready-to-use, Nichirei, Tokyo, Japan).

Histologically, the tumor was located in the lamina propria mucosae and tela mucosa (Fig. 2). In the lamina propria mucosae, in addition to neoplastic cell proliferation, formation of small clumps of bone tissue was prominent

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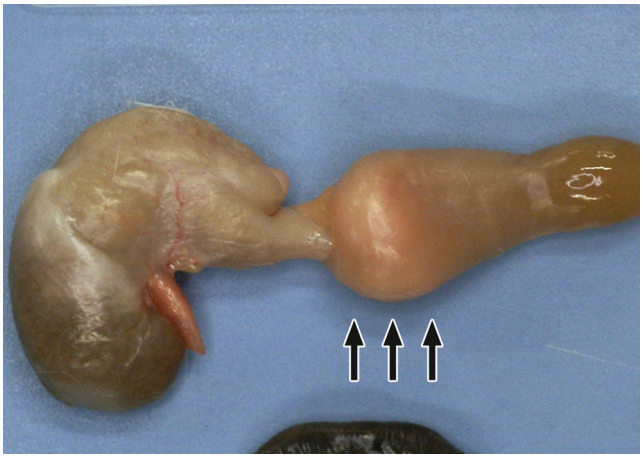

Fig. 1. Macroscopic appearance of the duodenum. A solid mass pushing up the tunica serosa (arrows) is palpable from the serous surface.

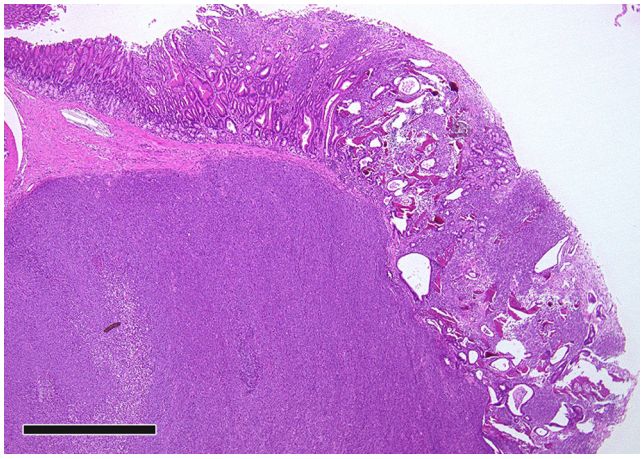

Fig. 2. Microscopic features of the tumor in the duodenal wall. The tumor is located in the lamina propria mucosae and tela mucosa. HE stain. Bar = 1,000 μm.

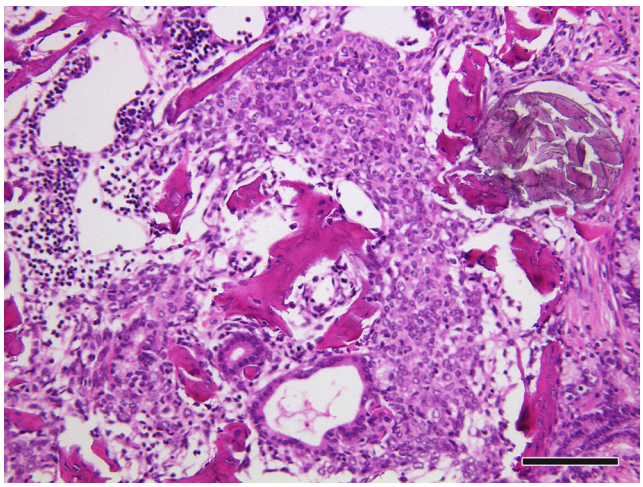

Fig. 3. A part of the lamina propria mucosae. Proliferation of polygonal cells and formation of small clumps of bone tissue in the lamina propria mucosae. HE stain. Bar = 100 μm.

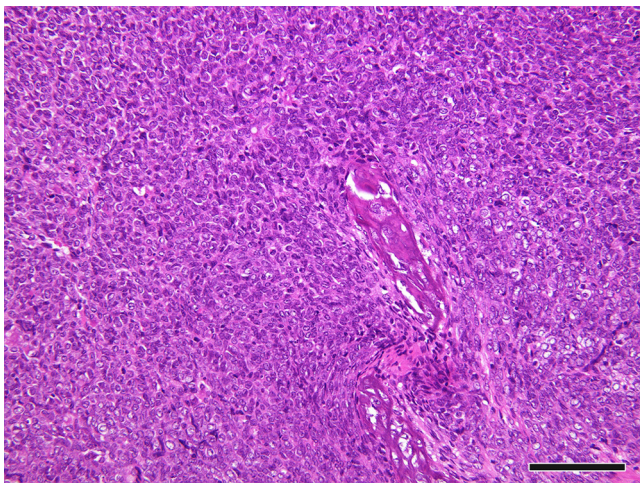

Fig. 4. A part of the tela mucosa. Marked proliferation of polygonal cells and spindle-shaped cells together with a small amount of osteoid and a small clump of bone tissue in the tela mucosa. HE stain. Bar = 100 μm.

(Figs. 2 and 3). The mucosal surface became flattened with disappearance of intestinal villi, and the remaining intestinal crypts generally showed luminal dilatation (Fig. 2). Neoplastic cells proliferated densely into the tela mucosa through the lamina muscularis mucosae, and replaced almost all of the normal tissue components including duodenal glands (Fig. 2). Neoplastic cells partially infiltrated into the tunica muscularis but not into the tunica serosa.

Neoplastic cells consisted of polygonal cells suggesting atypical osteoblasts and spindle-shaped cells, and polygonal cells were predominant (Figs. 3 and 4). Both types of neoplastic cells generally had a scanty basophilic or faintly eosinophilic cytoplasm and a pleomorphic plump nucleus (Figs. 3 and 4), and they occasionally showed mitosis. A few multinucleated cells resembling osteoclasts were also found. In the tela mucosa, a small amount of osteoid and a small clump of mature bone tissue also sporadically formed in the area of dense neoplastic cell proliferation (Fig. 4). In addition, chondroid elements were not identified morphologically in either area of neoplastic cell proliferation. Neither invasion of vessels in the duodenum nor metastasis to distant organs was observed.

Immunohistochemically, both polygonal and spindle-shaped neoplastic cells were positive for anti-osteocalcin, osteonectin, vimentin, and S-100 protein (Fig. 5), and the latter were also occasionally positive for α-SMA (Fig. 5). In addition, a few Iba-1-positive osteoclast-like multinucleated cells were found intermingled with neoplastic cells (Fig. 5). Such immuno-positive neoplastic cells were mainly observed around small clumps of bone tissue, especially in

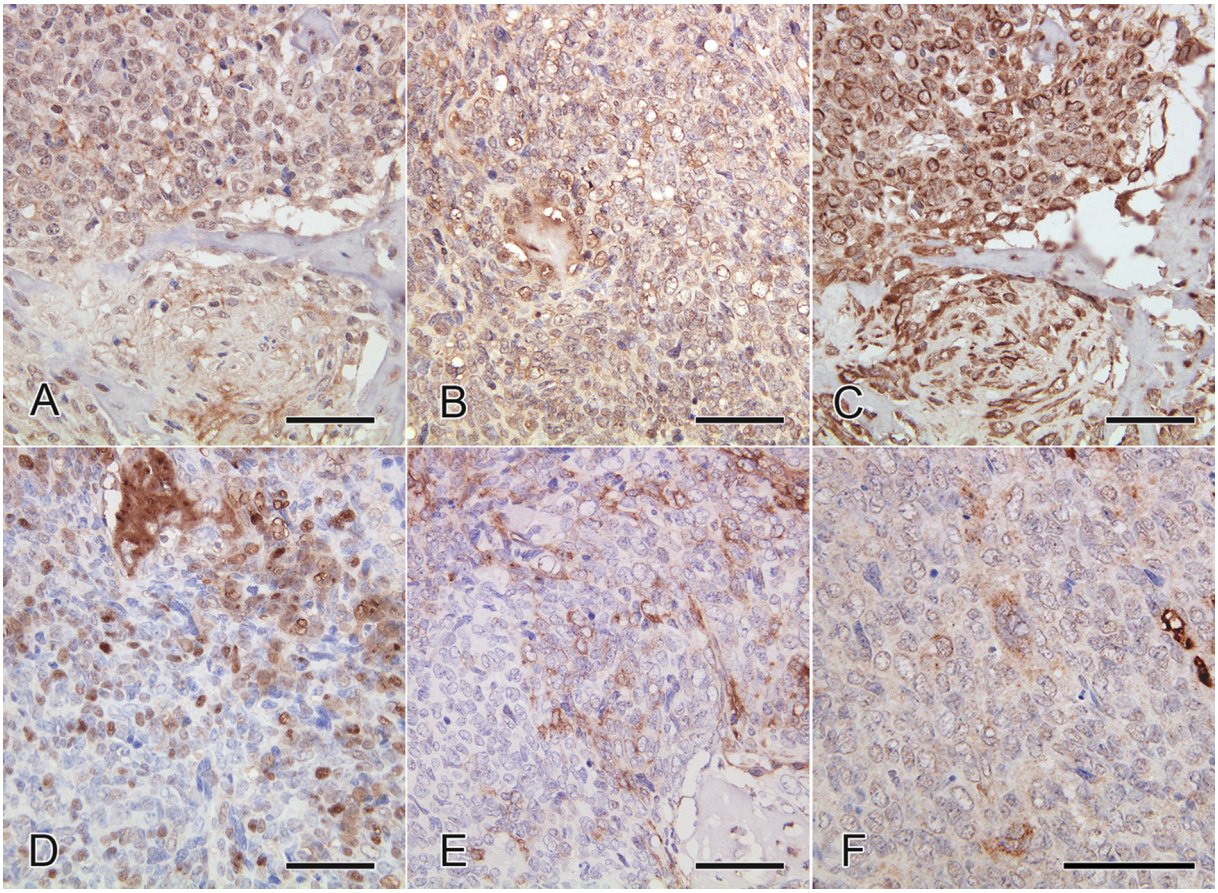

Fig. 5. Immunohistochemistry. Positive reactions for A) osteocalcin, B) osteonectin, C) vimentin, and D) S-100 protein in polygonal and spindle-shaped cells, E) α-SMA in spindle-shaped cells, and F) Iba-1 in multinucleated cells. Bar = 50 µm.

the lamina propria mucosae, except for those that were vimentin positive.

Osteocalcin and osteonectin are well-known reliable markers for osteogenic tumors, and some extraskeletal osteosarcomas are said to positively react with anti-vimentin, S-100 protein, and α-SMA antibodies[2, 3, 5, 6]. Judging from the abovementioned results, the present tumor was diagnosed as an extraskeletal osteosarcoma originating from the duodenum. In addition, the cause of demise of this animal was considered to be intestinal obstruction caused by the tumor.

Mouse osteosarcoma is classified into the following eight subtypes depending upon the morphologic characteristics of the tumor[8]: 1) the eburnating type, which is mainly composed of osteoma-like tumor osteoid/bone but with pleomorphic and infiltrating cells at the tumor periphery; 2) the osteoblastic type, which is composed of neoplastic osteoblasts and highly differentiated with varying amounts of osteoid and bone tissue; 3) the fibroblastic type, which is composed of spindle-shaped cells simulating a pattern of fibrosarcoma and a variable amount of osteoid; 4) the osteoclastic type, which is predominantly composed of osteoclast-like giant cells; 5) the chondroblastic type, which is composed of immature cartilage and bone; 6) the vascular type, which is composed of large blood-filled sinuses with variable amounts of osteoid and tumor cells; 7) the anaplastic type, which is composed of poorly differentiated pleomorphic tumor cells with scanty osteoid formation; and 8) the mixed type, which is composed of a combination of two or more histologic types. Judging from the abovementioned histological findings, the present osteosarcoma was considered to be the osteoblastic type.

In conclusion, this paper revealed the morphological and immunohistochemical characterization of the present tumor and described the first case of spontaneous duodenal extraskeletal osteosarcoma in a mouse.

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