*J Toxicol Pathol 2011; **24**: 75–79*

Case Report

Extraskeletal Osteosarcoma with Pulmonary Metastasis in a Female F344 Rat

Mika Nagaike[1], Kimie Sakai[1], Seiichiro Tsuchiya[1], Fumi Shimada[1], Kosei Inui[1], and Mamoru Uratani[1]

[1] Safety Science Laboratory, Central Research Institute, Ishihara Sangyo Kaisha Ltd., 2-3-1 Nishishibukawa, Kusatsu-shi, Shiga 525-0025, Japan

Abstract: A subcutaneous mass in the right femoral region of a female F344 Slc/N rat was examined histopathologically. At 83 weeks of age, the animal showed symptoms of severe anemia and nasal bleeding. Necropsy revealed that the mass had invaded the skeletal muscles but did not affect the bones. Multicentric nodules were also observed in the lung. Histopathology revealed a sheet-like growth pattern of polygonal tumor cells with round or comma-shaped nuclei and pale eosinophilic cytoplasm. Osteoid tissue was observed in not only the original lesion but also the metastatic foci in the lung. Each tumor cell was surrounded by argentophil fibers and few collagen fibers. Immunohistochemically, the tumor cells were positive for proliferating cell nuclear antigen (PCNA), vimentin, osterix and osteocalcin, but negative for keratin, S-100, von Willebrand factor, CD-31, CD-34, desmin, α-smooth muscle actin, lysozyme, α1-antitrypsin and rat malignant fibrous histiocytoma (MFH) antigen. CD-68-positive cells were considered to be infiltrated macrophages because they were negative for PCNA. On the basis of these findings, we diagnosed the present case as extraskeletal osteosarcoma. (DOI: 10.1293/tox.24.75; J Toxicol Pathol 2011; **24**: 75–79)

Key words: extraskeletal osteosarcoma, rat, subcutaneous, osteoid, osterix, osteocalcin

Extraskeletal osteosarcoma (ESO), a malignant mesenchymal neoplasm, produces osseous tissue that is not attached to the skeleton in humans[1]. ESOs have also been reported in dogs, cats[2], hamsters[3], rabbits[4] and rats[5–10]. We encountered a case of spontaneous ESO with pulmonary metastasis in an aged female F344 rat.

The present case was a female F344 Slc/N rat (Japan SLC, Inc., Hamamatsu, Japan) that was a subject in the control group of a 2 year carcinogenicity study. The animal was housed individually in a wire-mesh stainless steel cage placed on a movable stainless steel rack (CLEA Japan, Inc., Tokyo, Japan) in a semi-barrier-sustained animal room with a controlled environment (temperature, 22 ± 2°C; humidity, 55 ± 10%) and with illumination set with lights on at 07:00 and off at 19:00. Certified MF diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and local tap water (Kusatsu, Shiga) were freely supplied from a stainless steel basket and an automated water supply, respectively. During the study period, morbidity and mortality were checked at least twice a day except during public holidays. The treatment and handling of the animal conformed to the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006).

A palpable subcutaneous mass was noted in the right femoral region from 79 weeks of age. At 83 weeks, the animal was killed in extremis because of its unfavorable prognosis due to a fast decrease in body weight (–41 g from 4 weeks before), emaciation, anemia and nasal bleeding. While the rat was under deep anesthesia from intraperitoneal treatment with pentobarbital, blood was collected from the right ventricle. The animal was euthanized by exsanguination after the aorta and vena cava were cut, and then a complete necropsy was performed. The subcutaneous mass and all organs and tissues required by the toxicity test guidelines were sampled and fixed in 10% neutral buffered formalin.

At necropsy, the size of the mass was 4 × 3 × 3 cm. The surface was ulcerated, and the bottom side invaded the surrounding skeletal muscles. However, there was no connection with the neighboring bones. The cut surface of the mass revealed a whitish solid area that included many cysts containing bloody fluid and necrotic areas (Fig. 1a). In the lung, multicentric whitish nodules with hemorrhagic areas were observed (Fig. 1b). The trachea and nasal cavity were filled with a bloody foamy fluid. The stomach was empty except for black fluid that was thought to be swallowed hemoptysis. The liver and spleen were slightly enlarged. Petechiae were

Received: 29 October 2010, Accepted: 26 November 2010
Mailing address: Mika Nagaike, Safety Science Laboratory, Central Research Institute, Ishihara Sangyo Kaisha Ltd., 2-3-1 Nishishibukawa, Kusatsu-shi, Shiga 525-0025, Japan
TEL: 81-77-562-8924 FAX: 81-77-562-9587
E-mail: m-nagaike@iskweb.co.jp

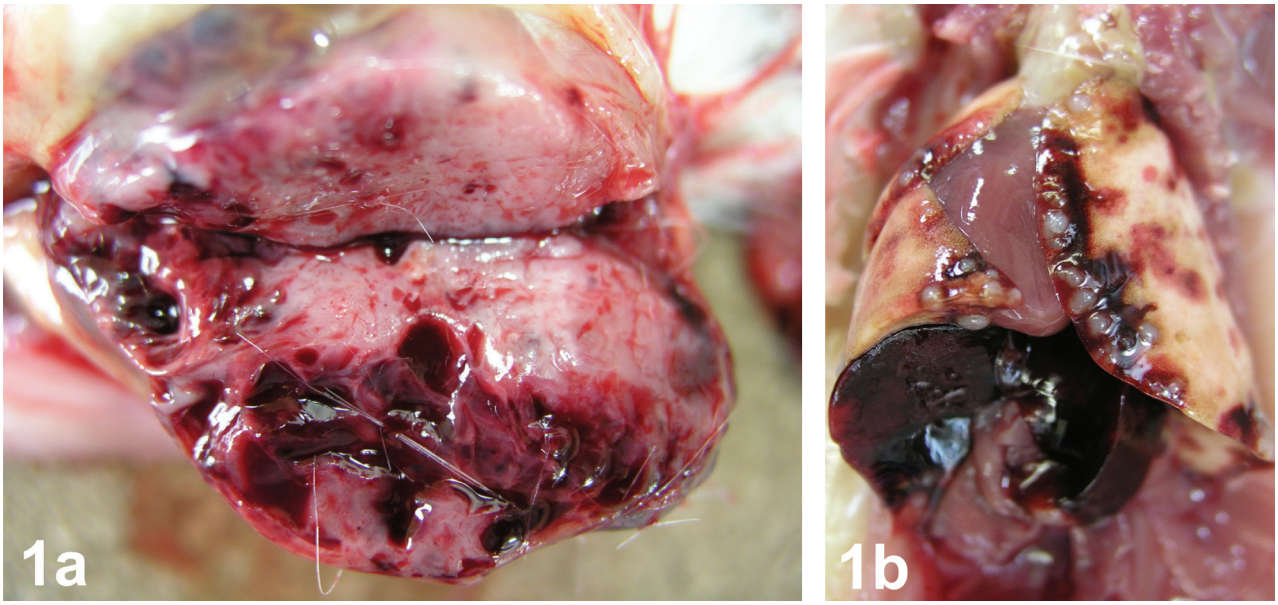

Fig. 1. a: Cut surface of the subcutaneous mass. The mass was composed of a solid area and cysts containing bloody fluid. b: Multicentric nodules in the lung. Hemorrhage is also noted.

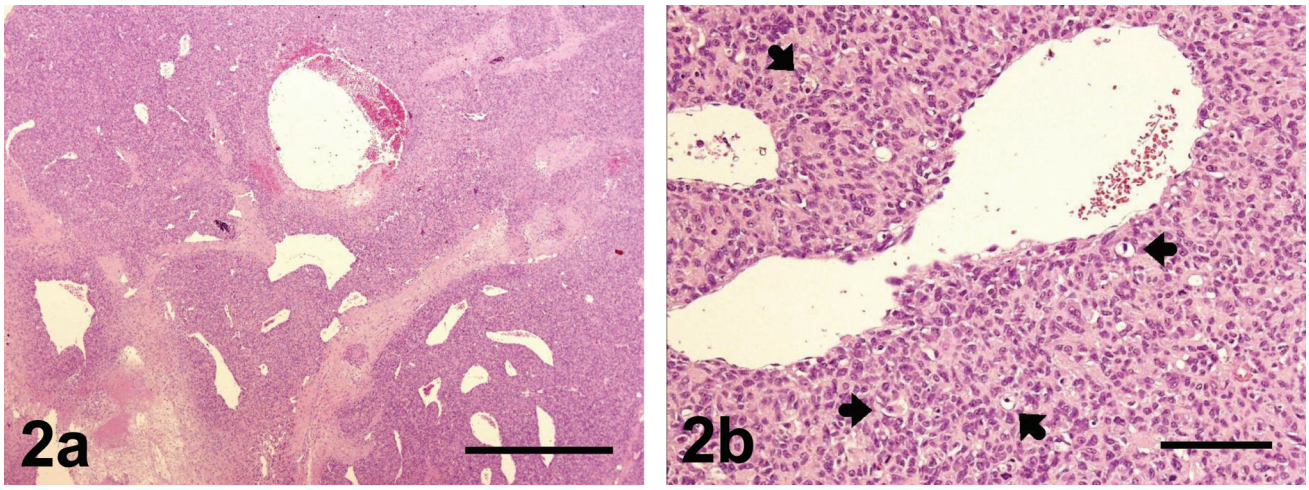

Fig. 2. a: Low magnification of the tumor tissue. There are many enlarged blood vessels, cysts filled with blood and necrotic areas. Bar = 1 mm. b: High magnification of the tumor tissue. Polygonal tumor cells reveal a sheet-like growth pattern around normal blood vessels. Arrowheads indicate vacuolated cells. Bar = 100 μm.

observed in the pituitary.

Hematological examination indicated a severe anemic condition (red blood cell (RBC) count, 1.33×10^6 cells/μL) associated with an increased number of white blood cells (WBCs) (14.4×10^3 cells/μL; lymphocytes, 91.8%). In the blood biochemical analysis, increased levels of plasma lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and creatine kinase (CK) suggested damage of the skeletal muscles by tumor invasion.

Routine histopathology was performed on all organs and tissues. Tissues were embedded in paraffin and then stained with hematoxylin and eosin (HE). In the HE sections, a subcutaneous mass was observed in the right femoral region. The mass was mainly composed of tumor cells that exhibited a sheet-like growth pattern (Fig. 2a). The macroscopic cysts were enlarged blood vessels and hematomas. Polygonal tumor cells had round or comma-shaped nuclei and clear eosinophilic cytoplasm (Fig. 2b). Mitosis was occasionally observed. Vacuolated cells were also frequently observed between the tumor cells (Fig. 2b, arrow-

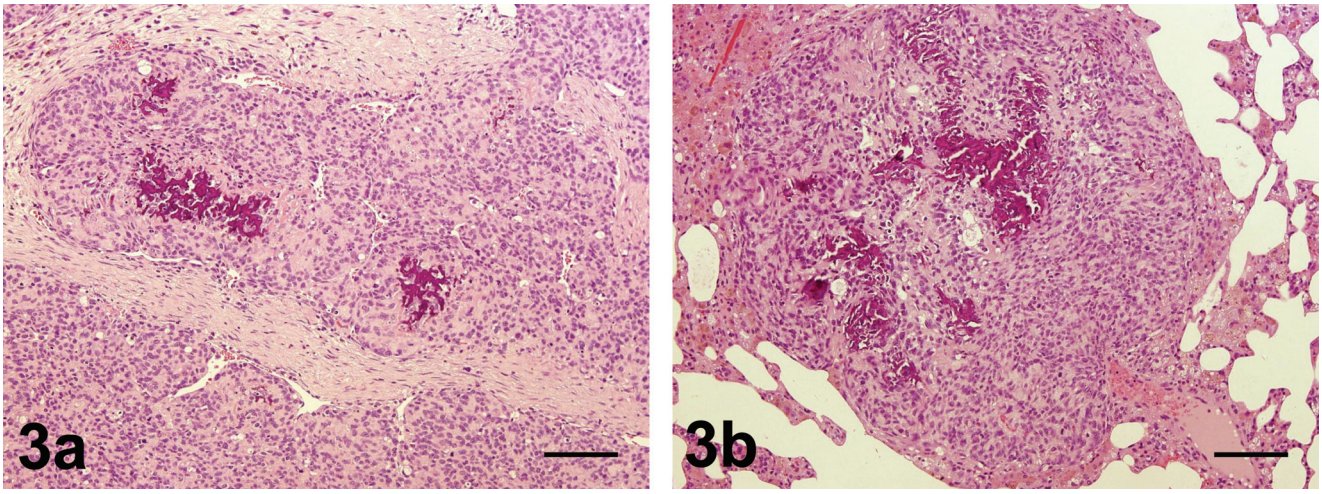

Fig. 3. Osteoid tissues are noted in the subcutaneous mass (a) and the pulmonary nodule (b). Bar = 100 μm.

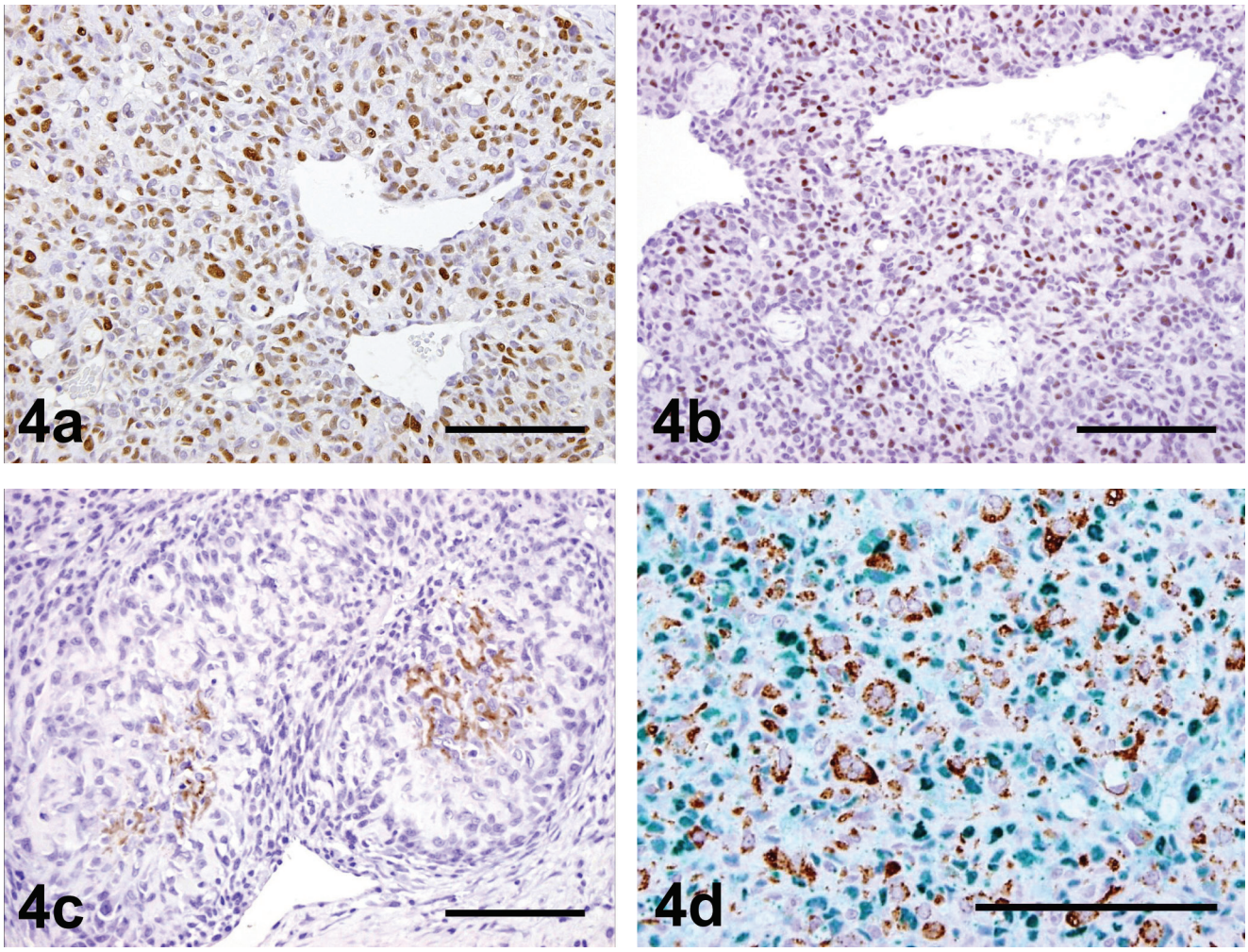

Fig. 4. Immunohistochemistry. The nuclei of the tumor cells are positive for proliferating cell nuclear antigen (PCNA) (a) and osterix (b). The osteoid area is positive for osteocalcin (c). CD68-positive cells (brown) are negative for PCNA (green). Bar = 100 μm.

Table 1. Summary of Immunohistochemical Results

Antigen	Results
Vimentin (V9)	+++
PCNA (PC10)	++
Osterix	+++
Osteocalcin (OCG3)	+ (osteoid)
Cytokeratin (AE1/AE3)	-
S-100	-
von Willebrand factor	-
CD31	-
CD34	-
α-Smooth muscle actin	-
Desmin	-
α-Antitrypsin	-
Rat MFH (A3)	-
PCNA & CD68 double staining	CD68-positive cells were negative for PCNA
Lysozyme	-
	Hyaline droplets in the kidney, +
α2u-Globulin*	Hyaline droplets in the kidney, –

* examined only for the kidney. PCNA, proliferating cell nuclear antigen; MFH, malignant fibrous histiocytoma.

heads). In a few areas, osteoid components that were surrounded by tumor cells were observed (Fig. 3a). A vascular structure lined by normal endothelial cells was noted as the interstitial component, and hemorrhagic and necrotic areas indicated circulatory failure in the tumor mass. The macroscopic nodules in the lung were composed of tumor cells associated with osteoid tissue (Fig. 3b), and these were considered to be the metastatic foci. Metastasis was not observed except for in the lung. Hyaline droplets were deposited in the proximal tubular cells, mainly at the S2 portion of the cortex in the kidney. Extramedullary hematopoiesis in the spleen was observed and considered to be an adaptive reaction to hemorrhage. Observed periportal hepatocellular fatty changes suggested that this animal had been fasting. A small focus of pituitary adenoma in the pars distalis and a slight change of hyperplasia in the mammary gland were incidental lesions.

In regard to special stainings of the tumor sections, Masson's trichrome staining revealed few collagen fibers in the neoplasm, whereas Watanabe's silver staining showed that each tumor cell was randomly surrounded by argentophil fibers. There was no positive labeling for Oil Red O or for the periodic acid Schiff (PAS) reaction. Immunohistochemically, tumor cells revealed strong positive labeling for anti-proliferating cell nuclear antigen (PCNA; PC10, monoclonal, ready-to-use; Dako, Denmark; Fig. 4a) and anti-vimentin (V9, monoclonal, ready-to-use; Dako). The nuclei of most tumor cells were positively stained for osterix (polyclonal, 1:200; Abcam, U.K.; Fig. 4b), which is a regulatory protein of bone cell differentiation[11,12]. All osseous areas were positive for osteocalcin (OCG3, monoclonal, 1:100; Abcam; Fig. 4c), a regulating factor for osteogenesis that is produced by osteoblasts[13,14]. These findings strongly suggested that the tumor cells had differentiated into osseous tissue. Vacuolated round cells that were scattered in the tumor were positive for CD-68 (ED-1, monoclonal, 1:100; Acris Antibodies, Germany) but negative for PCNA when double stained (Fig. 4d). Therefore, most of them were considered to be infiltrated macrophages rather than osteoclast-like differentiations. Staining for other markers, including S-100 (polyclonal, ready-to-use; Dako), von Willebrand factor (factor VIII-related antigen, polyclonal, ready-to-use; Dako), CD-31 (polyclonal, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), CD-34 (polyclonal, 1:100; R&D Systems,, Minneapolis, MN, U.S.A.), desmin (monoclonal, ready-to-use; Dako), α-smooth muscle actin (monoclonal, ready-to-use; Dako), lysozyme (polyclonal, 1:100; Progen, Germany), α1-antitrypsin (polyclonal, 1:50; GeneTex, Irvine, CA, U.S.A.), and rat malignant fibrous histiocytoma (MFH) antigen (A3, monoclonal, 1:50; TransGenic, Kumamoto, Japan), was negative (Table 1). Hyaline droplets in the kidney were positive for lysozyme (1:100) but negative for α2u-globulin (polyclonal, 1:50; R&D Systems).

On the basis of these findings, we diagnosed the present case as extraskeletal osteosarcoma due to osseous differentiation and no association with normal bones. Osteosarcomas are classified into 5 types in rats: osteoplastic, fibroblastic, osteoblastic, telangiectatic and compound[15]. The present case may be classified as the osteoblastic type. In humans, osteosarcomas are mainly observed in younger generations, but ESOs can be encountered in aged groups, and these cases are occasionally metastatic[1]. In addition, in rats, pulmonary metastasis is common in osteosarcomas that originate from the bone[15], but this was not noted in some previous cases of ESO in rats[5,8–10]. This evidence indicates the high malignancy of the present case.

Acknowledgments: We sincerely thank Ms. Naoko Hongo for her excellent technical assistance in histopathology and the members of the Kansai Conference of Toxicologic Pathology (KCTP) for their kind advice.

References

1. Weiss SW, and Goldblum JR. Osseous soft tissue tumors. In: Enzinger and Weiss's Soft Tissue Tumors. 4th ed. SW Weiss, and JR Goldblum (eds). Mosby, St. Louis. MO. 1389–1418. 2001.
2. Thompson KG, and Pool RR. Tumors of bones. In: Tumors in Domestic Animals. 4th ed. DJ Meuten (ed). Iowa State Press, Iowa. 245–318. 2002.
3. Madarame H, Itoh A, Hirose M, and Ogirara K. Spontaneous extraskeletal osteosarcoma of subcutis in Djungarian hamsters (*Phodopus sungorus*): report of two cases. J Vet

Med A Physiol Pathol Clin Med. **51**: 232–236. 2004.[Medline]
4. Renfrew H, Rest JR, and Holden AR. Extraskeletal fibroblastic osteosarcoma in a rabbit (*Oryctolagus cuniclus*). J Small Anim Pract. **42**: 456–458. 2001.[Medline] [CrossRef]
5. Minato Y, Yamamura T, Takada H, Kojima A, Imaizumi K, Wada I, Takeshita M, and Okaniwa A. An extraskeletal osteosarcoma in an aged rat. Jpn J Vet Sci. **50**: 259–261. 1987.[Medline]
6. Elwel MR and McConnell. Small and large intestine. In: Pathology of the Fischer Rat, Reference and Atlas. GA Boorman, SL Eustis, MR Elwell, MCA Montgomery, and WF MacKenzie (eds). Academic Press. San Diego. CA. 43–61. 1990.
7. Stefanski SA, Elwel MR, and Stromberg PC. Spleen, lymph nodes, and Thymus. In: Pathology of the Fischer Rat, Reference and Atlas. GA Boorman, SL Eustis, MR Elwell, MCA Montgomery, and WF MacKenzie (eds). Academic Press. San Diego. CA. 381–393. 1990.
8. Pace V, Persohn E, and Hieder K. Spontaneous osteosarcoma of the meninges in an albino rat. Vet Pathl. **32**: 204–207. 1995.[Medline] [CrossRef]
9. Yoshizawa K, Matsumoto M, Oishi Y, and Nyska A. Extraskeletal osteosarcoma with cystic appearance in an aged Sprague Dawley rat. Toxicol Pathol. **33**: 762–765. 2005.[Medline] [CrossRef]
10. Okazaki S, Ando R, Matsushima K, Hashita T, and Tamura K. Spontaneous extraskeletal osteosarcoma in the stomach of an aged F344 rat. J Toxicol Pathol. **23**: 157–159. 2010. [CrossRef]
11. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, and de Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell. **108**: 17–29. 2002.[Medline] [CrossRef]
12. Gao Y, Jheon A, Nourkeyhani H, Kobayashi H, and Ganss B. Molecular cloning, structure, expression, and chromosomal localization of the human Osterix (SP7) gene. Gene. **341**: 101–110. 2004.[Medline] [CrossRef]
13. Hauschka PV, Lian JB, and Gallop PM. Direct identification of the calcium-binding amino acid, gamma-carboxyglutamate, in mineralized tissue. PNAS. **72**: 3925–3929. 1975.[Medline] [CrossRef]
14. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, and Karsenty G. Endocrine regulation of energy metabolism by the skeleton. Cell. **130**: 456–469. 2007.[Medline] [CrossRef]
15. Mohr U (ed). Soft tissue and musculoskeletal system. In: International Classification of Rodent Tumors, Part 1 — The Rat, International Agency for Research on Cancer, Lyon. 1992.