Case Report

Osteosarcomas in the Lungs of an ICR Mouse

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Abstract: Osteosarcomas were found in the lung of a 62-week-old ICR female mouse having malignant systemic lymphoma. Macroscopically, the firm white nodules were located in all lobes of the lungs. Microscopically, the multiple nodules were mainly composed of irregularly shaped bone/osteoid tissues and pleomorphic or spindle-shaped osteoblast-like cells. Most of the tumors were well-differentiated and bone/osteoid tissues were abundant in the middle of the tumors, while at the tumor periphery and in a few nodules tumor cells were dominant with thin trabeculae of osteoid. These cells had hypochromatic, irregularly oval nuclei with a few small nucleoli, and, furthermore, anisokaryosis and mitosis were observed. The tumor cells were also present in the blood vessels and bronchial tubes. Immunohistochemically, the cells were positive for proliferating cell nuclear antigen (PCNA) and vimentin, but negative for cytokeratin and S-100 protein. Ultrastructurally, the cells had prominent nucleoli and dilated rough endoplasmic reticula, and collagen fibers with high electron density particles (hydroxy apatite) were observed around the cells. These findings suggest that the present tumors were malignant osteogenic neoplasms, and we diagnosed these tumors as osteosarcomas. Based on the multifocal growth pattern and presence in the blood vessels, in spite of the primary site being unknown, the tumors were considered to be metastatic. (*J Toxicol Pathol* 2003; **16**: 113–116)

Key words: ICR mouse, lung, osteosarcoma, metastasis, spontaneous, malignant lymphoma

In mice, spontaneous osteosarcoma is a rare neoplasm^{1–4}. The incidence of skeletal osteosarcomas is 0.2% in both male (2 of 891) and female (2 of 890) control ICR mice⁵ and ranges from 0 to 0.5% for males and 0 to 6.7% for females in control B6C3F1 mice⁶. Osteosarcomas originate in the vertebral column, mandible, femur, sternum, pelvis, and scapula, and readily metastasize to the lungs and liver via the bloodstream in mice². In the lungs, some cases of primary osteosarcomas have been reported in humans⁷, but not in animals. In BALB/c female mice, it has been reported that 7 of 16 osteosarcomas metastasized to the lungs and 2 occurred from an unidentified primary site³. Recently, we found spontaneous osteosarcomas in the lung of an ICR female mouse without detection of skeletal osteosarcomas. In this report, we describe the histological features and pathological development of this tumor.

The present lesions were found in a 62-week-old female ICR mouse (Japan SLC Inc., Shizuoka, Japan) used as a vehicle control (0.5% carboxymethyl cellulose, gavage) in a carcinogenicity study. The mouse was individually housed in a taper-type bracket cage in a barrier-sustained room

controlled at a temperature of $23 \pm 2^\circ\text{C}$, $55 \pm 10\%$ relative humidity, an illumination time of 13 hours/day at an intensity of about 200 lucas, and 10 to 15 ventilation cycles per hour. The animal had free access to a radiosterilized (30 kGy, ⁶⁰Co- γ -ray) pellet diet (NMF: Oriental Yeast Co., Ltd.) and tap water supplied through the nozzle of an automatic water supplying apparatus.

Clinically, the animal exhibited pale, hematuria, depression of activity, lowered body temperature, decrease in fecal volume, body weight and food consumption, and, therefore, was euthanized under ether anesthesia at 62 weeks of age. Macroscopically, the firm white nodules, which measured from 1 to 4 mm in diameter, were located in all lobes of the lungs (Fig. 1). Bloody fluid of about 1.2 mL was also observed in the thoracic cavity. Enlargement of the spleen and lymph nodes (mediastinal, mesenteric, pancreatic, mandibular, renal, and internal iliac), and a nodule in the uterus were detected. Hematological and blood chemical examinations revealed a marked increase in white blood cell count ($67,200/\mu\text{L}$), a marked decrease in erythrocyte count ($111 \times 10^4/\mu\text{L}$), and a high AST value. In the blood smear examination, numerous abnormal lymphoblastic cells were observed.

Organs and tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at thickness of 4 μm , and stained with hematoxylin and eosin (HE). Tartrate-resistant acid phosphatase (TRAP) staining was performed on paraffin sections of the lungs. In addition,

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Fig. 1. Gross appearance. Firm white nodules are located in all lobes of the lungs.

Fig. 2. Tumor is mainly composed of irregularly shaped bone/osteoid tissues. The border of tumor is indistinct and compression to the surrounding tissue is observed. HE stain. × 56.

Fig. 3. In the middle of tumor, osseous matrices are abundant. HE stain. × 220.

Fig. 4. At the tumor periphery, pleomorphic or spindle-shaped tumor cells are relatively prominent. HE stain. × 220.

Fig. 5. In a high cell density area, pleomorphic tumor cells with hypochromatic nuclei, anisokaryosis, and mitoses (arrowheads) are observed with thin osteoid trabeculae. Osteoclast-like cells are present (arrow). HE stain. × 220.

Fig. 6. Tumor cells are observed in the blood vessel (arrowheads). HE stain. × 220.

immunohistochemical examination for proliferating cell nuclear antigen (PCNA) (DAKO Japan Co., Ltd., Tokyo, Japan), vimentin (ICN Pharmaceuticals, Inc., USA), cytokeratin AE1+AE3 (DAKO Japan), S-100 protein (DAKO Japan), osteonectin (Cosmo Bio Co., Ltd., Tokyo, Japan), and alpha-smooth muscle actin (alpha-SMA) (Sigma-Aldrich Japan K.K., Tokyo, Japan) were performed on paraffin sections of the lungs using a standard labeled streptavidin biotin technique (DAKO LSAB/LSAB2 Kit, DAKO Japan). An electron microscopic examination was also performed using the formalin-fixed lungs.

Microscopically, malignant lymphoma cells were present in the lungs and many organs such as the spleen, thymus, lymph nodes, bone marrow, liver, kidneys, ovaries, stomach, colon, pancreas, urinary bladder, adrenal glands, pituitary gland, salivary glands, mammary glands, and Harderian glands. Cystic endometrial hyperplasia was also observed. In the lungs, multiple nodules were mainly composed of irregularly shaped bone/osteoid tissues and pleomorphic or spindle-shaped osteoblast-like cells, and the border of nodules was irregular. The dominance of bony tissue or tumor cells varied between individual nodules as well as in different portions of the same nodule. Most of the nodules were well-differentiated and the bone/osteoid tissues were extremely abundant (Fig. 2). In the middle of the nodules, osseous matrices were abundant with a small number of tumor cells having small and hyperchromatic nuclei (Fig. 3), while at the periphery pleomorphic tumor cells were prominent with relatively large and oval nuclei (Fig. 4). In a few nodules, tumor cells were dominant with thin trabeculae of osteoid and those cells had hypochromatic, irregularly oval nuclei with a few small nucleoli, and, furthermore, anisokaryosis and mitosis were moderately observed (Fig. 5). Multinucleated cells having a few nuclei were scattered in some nodules and were positive for TRAP staining, a marker of osteoclasts. The tumor cells were also present in the blood vessels (Fig. 6) and bronchial tubes. There were many lymphoma cells in and around the small vessels among the bone/osteoid trabeculae, which resembled marrow cavities, however, hematopoiesis was not observed. Immunohistochemically, the majority of tumor cells were positive for PCNA and slightly positive for vimentin, whereas they were negative for cytokeratin and S-100 protein, and equivocal for alpha-SMA and osteonectin. Ultrastructurally, the tumor cells had prominent nucleoli and dilated rough endoplasmic reticula. Other organelles were scanty. Collagen fibers with high electron density particles (hydroxy apatite) were observed around the cells.

The present lesions formed multiple nodules located in all lobes of the lungs of an ICR female mouse having malignant systemic lymphoma. The nodules were mainly composed of irregularly shaped bone/osteoid tissues and pleomorphic or spindle-shaped osteoblast-like cells. Immunohistochemical examination revealed that the tumor cells were markedly proliferative and were not of epithelial origin. Ultrastructural findings demonstrated that the tumor cells were osteoblasts in origin. Most of the tumors were

well-differentiated and the bone/osteoid formation was extremely prominent as if the nodules were osteomas or osseous metaplasias. Osteomas are benign neoplasms, composed of very dense bone and generally arise from the bone surface^{6,8}. Osteomas occurring in the lungs of a rodent have not been reported in the literature. Multiple osteomas have been reported in Him:OF1 mice, but these osteomas occurred at multiple sites of the skeleton and not in the lungs⁹. Pulmonary osseous metaplasias are non-neoplastic lesions and have occasionally been observed in mice^{10,11}. These lesions consist of deposits of osteoid with or without early mineralization and, generally the osteocytes and osteoblasts are not prominent without cellular atypia or mitosis. In the present case, areas of relatively high cell density were observed at the tumor periphery and in a few tumors, and the tumor cells exhibited cellular atypia, mitosis, and a high proliferation activity. In addition, the tumor cells invaded the bronchial tubes and blood vessels. These facts suggest that the present lesions were malignant osteogenic neoplasms. We, therefore, diagnosed the lesions as osteosarcomas.

Osteosarcomas in mice have been classified into eburnating, osteoblastic, fibroblastic, osteoclastic, chondroblastic, vascular, anaplastic, and mixed types^{6,8}. Based on this classification, the present case was considered to be an eburnating type. However, the grade of tumor differentiation varied between individual tumors and in different portions of the same tumor, and, generally, the bone/osteoid tissues were abundant in the middle of the tumors, while tumor cells were prominent at the tumor periphery. In the literature, it has been reported that osteosarcomas show a variety of histologic features^{3,6}, such as progressive maturation from newer to older portions of the tumor^{8,12}, zonation phenomenon where "older" (more productive) cells are centrally located and "younger" (less productive) cells are peripherally located⁶. Marrow cavities with hematopoietic tissue and osteoclasts may be present in older ossified areas, and similar maturation may occur even in metastases¹². The results of these reports correspond with the histologic features of the present case, and support our diagnosis of the lesions as osteosarcoma.

Osteosarcomas originate in the vertebral column, mandible, femur, sternum, pelvis, and scapula, and readily metastasize to the lungs and liver via the bloodstream². Osteogenic sarcomas are most likely to metastasize to the lungs⁴. The present osteosarcomas in the lungs showed a multifocal growth pattern and a proliferation in the blood vessels. These facts suggest that the present osteosarcomas are metastatic. However, the primary site of the neoplasm was not identified in the routine macroscopic or microscopic examination. No primary osteosarcomas of the lung in animals have been found in the literature. In humans, some cases of primary osteosarcomas of the lung have been reported⁷, however, in each case, the neoplasms occurred as a solitary nodule or two nodules, namely, the human cases were different from the present case which had multiple nodules. In BALB/c mice, 2 cases have been reported as

pulmonary metastases of osteosarcoma from an unidentified primary site³. It has been stated in the literature that there is no obvious correlation between histologic differentiation or tumor size and the probability of metastasis^{8,12}. Therefore, in spite of the primary site being unknown, the present osteosarcomas in the lungs were considered to be metastatic.

In conclusion, multiple nodules were observed in the lungs of an ICR female mouse having malignant systemic lymphoma. The nodules were diagnosed as osteosarcomas and, in spite of the primary site being unknown, they were considered to be metastatic.

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References

1. Albassam MA and Courtney CL. Nonneoplastic and neoplastic lesions of the bone. In: Pathobiology of the Aging Mouse, Vol. 2, U Mohr, DL Dungworth, CC Capen, WW Carlton, JP Sundberg, and JM Ward (eds), Washington DC: ILSI Press, 425–437, 1996.
2. Wadsworth PF. Classification of bone neoplasms and presentation of data on the incidences and types in C57BL/10J mice. In: Pathobiology of the Aging Mouse, Vol. 2, U Mohr, DL Dungworth, CC Capen, WW Carlton, JP Sundberg, and JM Ward (eds), Washington DC: ILSI Press, 439–443, 1996.
3. Frith CH, Johnson BP, and Highman B. Osteosarcomas in BALB/c female mice. *Lab Anim Sci* 1982; **32**: 60–63.
4. Frith CH, Littlefield NA, and Umholtz R. Incidence of pulmonary metastases for various neoplasms in BALB/cStCrIfC3H/Nctr female mice fed N-2-fluorenylacetamide. *J Natl Cancer Inst* 1981; **66**: 703–712.
5. Maita K, Hirano M, Harada T, Mitsumori K, Yoshida A, Takahashi K, Nakashima N, Kitazawa T, Enomoto A, Inui K, and Shirasu Y. Mortality, major cause of moribundity, and spontaneous tumors in CD-1 mice. *Toxicol Pathol* 1988; **16**: 340–349.
6. Long PH and Leininger JR. Bones, joints, and synovia. In: Pathology of the mouse, Maronpot RR (ed), Vienna, IL: Cache River Press, 645–678, 1999.
7. Loose JH, El-Naggar AK, Ro JY, Huang WL, McMurtrey MJ, and Ayala AG. Primary osteosarcoma of the lung. *J Thorac Cardiovasc Surg* 1990; **100**: 867–873.
8. Ernst H, Long PH, Wadsworth PF, Leininger JR, Reiland S, and Konishi Y. Skeletal system and teeth. In: International Classification of Rodent Tumors: The Mouse, U Mohr (ed), Germany: Springer-Verlag Berlin Heidelberg, 389–415, 2001.
9. Höger H, Gialamas J, and Jelinek F. Multiple osteomas in mice. *Vet Pathol* 1994; **31**: 429–434.
10. Dixon D, Herbert RA, Sills RC, and Boorman GA. Lungs, pleura, and mediastinum. In: Pathology of the mouse, Maronpot RR (ed), Vienna, IL: Cache River Press, 293–332, 1999.
11. Ernst H, Dungworth DL, Kamino K, Rittinghausen S, and Mohr U. Nonneoplastic lesions in the lungs. In: Pathobiology of the Aging Mouse, Vol. 1, U Mohr, DL Dungworth, CC Capen, WW Carlton, JP Sundberg, and JM Ward (eds), Washington DC: ILSI Press, 281–300, 1996.
12. Nilsson A and Stanton MF. Tumours of the bone. In: Pathology of Tumours in Laboratory Animals, Vol. 2, Tumours of the mouse, 2nd ed., VS Turusov and U Mohr (eds), Lyon, France: IARC, 681–700, 1994.