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Original contribution

Lymphatics and bone☆

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Summary There is controversy regarding whether lymphatic vessels are present or absent in bone. Although lymphangiomas have been described in bone, lymphatic vessels have not been identified morphologically with certainty in any other benign or malignant bone tumors or in normal human bone. In this study, we determined by immunohistochemistry, using 2 specific lymphatic endothelial cell markers, LYVE-1 and podoplanin, whether lymphatics are present in normal bone and a wide range of primary and secondary bone neoplasms. In normal bone, LYVE-1$^+$/podoplanin$^+$ lymphatic vessels were not identified in cortical or cancellous bone but were seen in connective tissue overlying the periosteum. With the exception of lymphangioma, Gorham-Stout disease, and hemangioendothelioma, primary benign and malignant bone tumors (as well as secondary carcinomas) that were confined to bone did not contain lymphatic vessels. Primary and secondary bone tumors that had extended through the bone cortex contained LYVE-1$^+$/podoplanin$^+$ lymphatic vessels that seemed to extend for a short distance from surrounding soft tissues into the tumor. Three cases of osteosarcoma that had extended through the bone cortex and had lymph node metastases were all found to contain lymphatic vessels within the tumor. These results indicate that the lymphatic circulation is unlikely to play a role in bone fluid transport in normal bone and that lymphatic vessels are absent from most primary and secondary tumors confined to bone. These findings also suggest that lymphangiogenesis is not involved in the disease progression of most primary bone tumors and that carcinomatous metastasis to bone does not occur via lymphatics.

1. Introduction

The lymphatic circulation is a branching system of thin-walled vessels lined by endothelial cells [1,2]. Lymphatic capillaries are thin-walled vessels lined by flattened endothelial cells; larger lymphatic vessels also have a thin

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coat of smooth muscle in the vessel wall. Several factors regulating the development and function of lymphatic vessels have been identified. This has led to the discovery of phenotypic markers, such as podoplanin, prox-1, LYVE-1, and VEGFR3, which are relatively specific for lymphatic endothelial cells [3-7]; these markers permit identification of lymphatic vessels in normal and pathological tissues and allow histologic distinction between lymphatic vessels and blood vessels to be made with certainty.

Lymphatic vessels are believed to be absent from several tissues, including the eye and brain [8,9]. Bone has an extensive blood vessel network that includes numerous dilated thin-walled vessels (sinusoids), which are difficult to distinguish from lymphatics morphologically, and there are conflicting reports regarding the presence or absence of lymphatics in bone [10-15]. Some histomorphological and tissue perfusion studies suggest that lymphatics or "pre-lymphatic vessels" are present in the periosteum and around blood vessels within cortical Haversian systems [12,13,16-21]. The only pathological condition in which lymphatic vessels in bone have been identified morphologically with some certainty is that of lymphangioma or lymphangiomatosis of bone [22,23]. Whether other vascular tumors of bone contain lymphatic vessels has not been established with certainty.

One of the major obstacles to resolving the controversy regarding the presence or absence of lymphatics in bone is the difficulty of distinguishing lymphatic vessels from blood vessels with certainty. In this study, we have used 2 highly specific lymphatic endothelial cell markers, podoplanin and LYVE-1 [3,4], to establish whether lymphatics are present or absent in normal bone. We have also examined a wide range of primary and secondary neoplasms of bone not only to identify whether lymphatic vessels are present in these tumors but also to determine whether lymphangiogenesis and the lymphatic circulation play a role in tumor progression.

2. Materials and methods

Samples of normal bone and bone affected by a wide range of neoplasms were obtained from the Histopathology Departments of the Nuffield Orthopaedic Centre, Oxford; the Royal Orthopaedic Hospital, Birmingham; the N. Goormaghtigh Institut, Ghent; and the Leiden University Medical Centre. Normal bone specimens examined included 6 femora, 4 tibiae, 4 metatarsal bones, 4 humeri, 4 radii, and 4 digital phalanges derived from amputation specimens. Morphologically normal bone specimens of the vertebra (2 specimens), skull (6 specimens), jaw (4 specimens), facial bone (2 specimens), sternum (1 specimen), and clavicle (1 specimen) were also examined. The number and nature of the primary and secondary bone tumors examined in this study are shown in Table 1. Three cases of osteosarcoma with known lymph node metastasis were included in this study.

Table 1 Neoplastic lesions of bone examined in this study

Primary bone tumors and tumor-like lesions
Osteoma (2), osteoid osteoma (2), osteoblastoma (3), osteosarcoma [a] (21), osteochondroma (2), enchondroma (2), chondroblastoma (8), conventional chondrosarcoma (9), clear cell chondrosarcoma (1), mesenchymal chondrosarcoma (1), dedifferentiated chondrosarcoma (1), giant cell tumor of bone (10), giant cell reparative granuloma of jaw (2), Ewing sarcoma (10), myeloma (2), lymphoma (10), leiomyosarcoma (1), malignant fibrous histiocytoma (4), nonossifying fibroma (2), fibrous dysplasia (3), fibro-osseous dysplasia (2), simple bone cyst (3), aneurysmal bone cyst (3), chronic lymphocytic leukemia (2), hemangioma (16), Gorham-Stout disease (2), hemangioendothelioma (2), adamantinoma (2), Langerhans cell histiocytosis (3), chordoma (4), Paget sarcoma (1)
Secondary bone tumors
Metastatic carcinoma (37)
(Primary origin: kidney [8], breast [18], prostate [6], nasopharyngeal [1], lung [2], colon [2])
Metastatic melanoma (2)

NOTE. The number of tumors examined is shown in parenthesis.
[a] Three of the osteosarcomas showed extraosseous extension and had lymph node metastases.

All samples of bone tissue were fixed in formalin and, after decalcification, processed routinely and embedded in paraffin wax. Five-micrometer sections were cut onto silane-coated slides (Surgipath, Peterborough, UK); the sections were incubated at 37°C for 24 hours before staining to improve tissue adhesion.

All immunohistochemical staining was performed using an indirect immunoperoxidase technique (ChemMate Envision; Dako, Ely, UK). Tissue sections were dewaxed and rehydrated by successive immersion in xylene, graded ethanol, and water. Antigen retrieval was performed by microwave treatment (700 W, 2 × 4 minutes) in Target Retrieval Solution (Dako, UK). Endogenous peroxidase was blocked by 0.2% (vol/vol) hydrogen peroxide in 80% ethanol and protein block serum before 30 minutes of incubation with mouse anti–LYVE-1 and antipodoplanin monoclonal antibodies [3,4]. Immunohistochemistry was carried out using the monoclonal antibodies JC70 and QBend10 (Dako, Ely, UK) directed against the vascular endothelial cell markers CD31 and CD34, respectively. Antigens were detected by incubation with labeled polymer and diaminobenzidine. The sections were then counterstained with hematoxylin, dehydrated, cleared, and mounted. Sections of normal skin and lymph node were used as positive control tissue; these contained both lymphatic and blood vessels. Negative controls consisted of sections stained by the above immunohistochemical technique using primary antibody diluent alone or substitution of an appropriate irrelevant antibody for the tumor under investigation (eg, MNF116 anticytokeratin antibody [Dako] for primary bone-forming neoplasms).