Osseous Metaplasia in the Mesenteric Lymph Node with Metastatic Cholangiocarcinoma in a Cow
S. Ohfuji
Department of Histopathology, Diagnostic Animal Pathology Office, Hokkaido, Japan

Abstract
Introduction: Osseous metaplasia in extraosseous sites associated with neoplasm is uncommon. The pathogenic mechanism remains poorly understood. Occurrence of osseous metaplasia within the lymph node affected with metastatic cancer has not been reported in detail in humans and animals yet, to the author’s knowledge.

Presentation of Case: A Holstein-Friesian dairy cow, aged 8 years and 9 months, was slaughtered for food and revealed multiple neoplastic nodules in the liver and many other visceral organs and tissues. Histopathology and immunohistochemistry made a diagnosis of intrahepatic cholangiocarcinoma displaying an aggressive behavior with widespread metastasis. A mesenteric lymph node with metastatic cholangiocarcinoma had extensive areas of osseous metaplasia, exhibiting well-differentiated bone trabeculae dependent on the balance between osteoblastic proliferation and osteoclastic bone resorption. Necrotic areas in this metastatic nest underwent a response by macrophages and multinucleated giant cells in the stromal connective tissue that showed prominent fibroblastic proliferation and abundant capillaries, suggesting that high activity of mesenchymal cells resident at the lymph node was implicated in the development of bone trabeculae.

Conclusion: This appears to be the first to report osseous metaplasia in the lymph node with metastatic cancer. Although a precise explanation for its formation remains to be determined, this bovine case may be useful in the future when researching human cancer associated with osseous metaplasia.

Keywords: Bovine; Cholangiocarcinoma; Lymph node; Osseous metaplasia

Introduction
The term osseous metaplasia, heterotopic bone formation, or ectopic ossification has been used to designate nonneoplastic bone formation in extraskeletal locations [1]. The incidence of osseous metaplasia within nonosseous differentiation neoplasm is rare in both humans and animals, and its biologic significance is poorly understood. Osseous and/or cartilaginous metaplasia has previously been documented, for instance, in ovarian adenocarcinoma [2] or cystadenoma [3], renal cell carcinoma [4, 5], transitional carcinoma of the ureter [6], breast cancer [7], spindle cell carcinoma [8], colonic adenoma [9, 10], rectal cancer [11], squamous cell carcinoma [12], and diffuse large B-cell lymphoma [13].
reports of osseous metaplasia associated with neoplasm, including those describing a horse with vaccine-associated fibrosarcoma [14], a horse with colonic adenocarcinoma [15], a ferret with pancreatic adenocarcinoma [16], a llama with mammary neoplasm [17], a cat with astrocytoma [18], a dog with fibroma [19], two dogs with cutaneous lipoma [20], and dogs with mammary, thyroid, or salivary carcinomas [1].

To the author’s knowledge, the occurrence of osseous metaplasia in any lymph node undergoing metastasis from cancer is what has never previously been reported in humans and any animal species. This report describes a case of spontaneous development of osseous metaplasia in the mesenteric lymph node that was affected with metastasis from cholangiocarcinoma in a cow.

Case Presentation

A Holstein-Friesian dairy cow, aged 8 years and 9 months, was slaughtered for human consumption at an abattoir. Postmortem examination revealed multiple, non-encapsulated, grayish-white, firm, tumor nodules (up to 3 cm in diameter) in the carcass, most prominently in the liver (Fig. 1). In the latter, tumors were scattered throughout all liver lobes, and a superficially located tightly adhered to the peritoneal surface of the diaphragm. Similar one or more tumors were recognized in the lung, pancreas, lymph nodes (hepatic, pancreaticoduodenal, mesenteric, tracheobronchial, and mediastinal), parietal pleura, peritoneal surface of the diaphragm, splenic and forestomach serosa, omentum majus, and supraorbital region. One of the mesenteric lymph nodes was enlarged, as in other affected lymph nodes, but firm with some areas gritty to feel on cutting. Its nodal architecture was totally replaced by tissue accompanied by massive discolored solid area (Fig. 1, inset). Other significant visceral lesions included fibrinous pericarditis and infarcted kidneys.

**Figure 1** Cut surface of the liver demonstrating many tumor nodules of varying sizes. Bar = 10 mm. Inset: Cut surface of the mesenteric lymph node representing complete distortion of nodal architecture due to the presence of metastatic tumor that shows discolored solid area (arrow). Bar = 5 mm.

Upon histopathological examination of formalin-fixed, paraffin-embedded, hematoxylin and eosin (HE)-stained sections, the hepatic neoplasm was consistent with intrahepatic cholangiocellular carcinoma. This neoplasm,
which invaded or compressed adjacent hepatic lobules, displayed acinar, tubular, or pseudorosette structures accompanied by abundant connective tissue stroma and areas of massive necrosis and hemorrhage (Fig. 2). Occasionally, the lumina of the tubules contained basophilic mucinous materials that stained positively with alcian blue (pH 2.5). Neoplastic cells were cuboidal or columnar had moderate amounts of basophilic cytoplasm and round-to-oval, vesicular nuclei of varying sizes, with dispersed coarse chromatin and one nucleolus (Fig. 2, inset). Mitotic activity was very high, exhibiting multiple (6-10) bizarre mitotic figures per high-magnification microscopic field (400 x). By immunohistochemistry using a streptavidin-biotin-immunoperoxidase method (EnVision+ kit, Dako, Glostrup, Denmark), the neoplastic cells demonstrated expression of cytokeratin-7 (CK7, monoclonal mouse, Dako, 1:50) and exhibited no immunoreactivity for human hepatic marker (Hepar 1, monoclonal mouse, Dako, 1: 50).

In the hepatic portal triads, not only biliary hyperplasia but also intravascular and intralymphangial tumor emboli were observed. Aggregates of lymphocytes were occasionally noted. The cranial mesenteric ganglion and the vagal nerve at the mediastinal site, as well as those extrahepatic organs and tissues, which were grossly identified to have tumors, were consistently involved with metastatic cholangiocellular carcinoma. In most of these affected organs and tissues, there were tumor emboli, with or without hyaline thrombi, in lymphatic and blood vessels, where neoplastic cells often proliferated with supporting connective tissue stroma that was continuous with the surface of these lumina.

Figure 2 Lesion of cholangiocarcinoma invading the surrounding hepatic lobules. Intravascular tumor embolus (arrow) is seen in the portal area. HE. Bar = 80 μm. Inset: Cuboidal neoplastic cells of cholangiocarcinoma form acinar structures. HE. Bar = 40 μm.

A mesenteric lymph node had markedly thickened fibrous capsule and was completely replaced by desmoplastic neoplasm that metastasized from primary cholangiocarcinoma, giving rise to the disappearance of nodal architecture. Marginal regions of necrotic foci in this metastatic cholangiocarcinoma frequently underwent a reparative response by large numbers of foamy macrophages and fewer numbers of foamy multinucleated giant cells of the foreign body type (Fig. 3). In addition, within and between tumor islands there were extensive areas representing formation of bone trabeculae, consistent with osseous metaplasia (Fig. 4). Each bone trabecula was well differentiated with evident proliferation of osteocytes embedded in eosinophilic bone matrix that demonstrated partial mineralization in some places. Usually, a single layer of osteoblasts or osteoclasts lined the bone trabeculae. Connective tissue stroma enclosing both tumor islands and bone trabeculae was edematous or, in some sites, myxomatous and displayed varying degrees of hemorrhage. There also were prominent proliferation of fibroblasts with occasional mitotic figures, abundant capillaries, and mild cellular infiltrates comprised of foamy macrophages, siderophages, erythrophages, lymphocytes, plasma cells, and eosinophils. Some macrophages phagocytosed...
greenish-brown pigments different from hemosiderin. Other microscopic lesions were as follows: chronic fibrinous pericarditis, pleuritis, and conjunctivitis; renal infarcts associated with hyaline thrombi in the interlobular arteries whose walls showed fibrinoid necrosis; and severe splenic hemosiderosis with extramedullary hematopoiesis.

Figure 3 Foamy macrophages and foamy giant cells (arrows) are present around necrotic area (*) of metastatic cholangiocarcinoma in the mesenteric lymph node. HE. Bar = 30 μm.

Figure 4 Feature of osteoclastic (arrows) bone resorption is evident in a metaplastic bone trabecula that has mature osteocytes and is surrounded by edematous, capillary-rich connective tissue stroma of metastatic cholangiocarcinoma (*) in the mesenteric lymph node. HE. Bar = 60 μm.
Discussion

Cholangiocarcinoma is the second most common primary hepatic malignancy after hepatocellular cancer [21, 22], showing an increased global incidence [23]. It has also been reported in many domestic animal species, including dogs, cats, sheep, cattle, horses, and goat [24-27]. Characteristics of the desmoplasia, mucin production, absence of the bile canaliculi, and CK7 immunolabeling of neoplastic cells distinguished the cholangiocarcinoma described in the present cow from an adenoid variant of hepatocellular carcinoma [25, 26]. Given the presence of high mitotic rate of neoplastic cells, the tendency to invade without encapsulation, the widespread necrosis/hemorrhage, and the high rate of extrahepatic metastasis, it is believed that this spontaneously occurring intrahepatic cholangiocarcinoma was highly malignant and likely had aggressive potential. Metastasis of cholangiocarcinoma, which is not uncommon in cattle [24, 25], was likely systemic one and spread not only via lymphatic and vascular route but also by abdominal serosal implantation, taking into account the presence of tumor emboli in the lymphatics and blood vessels and the macroscopic evidence of the adhesion of a superficial nodule of cholangiocarcinoma to the diaphragm.

More unique was the occurrence of distinct bone trabeculae in a mesenteric lymph node to which this cholangiocarcinoma metastasized. Bone tissue in any tumor has been thought to originate through two different processes: osseous differentiation tumor or osseous metaplasia [5]. In a previous study of pseudosarcoma of the mouth, this tumor, alternatively referred to as metastastic carcinoma or spindle cell carcinoma, was reported to metastasize to the neck and paratracheal lymph nodes and therein differentiate to bony and cartilaginous components as well as squamous epithelial pearls [28]. However, it is indeed unlikely that cholangiocarcinoma is potentially a metastatic neoplasm with osteoblastic differentiation. In the present bovine case, bone tissue itself lacked evidence of a neoplastic component, distinguishing it from osteoma or osteosarcoma. The term osseous metaplasia may be entirely correct for this case, because the process of bone formation was dependent on the balance between osteoblasts and osteoclasts, as seen in the normal skeleton [1]. In the latter, the coordinated activity of osteoblasts and osteoclasts is mediated by an interaction between a variety of systemic hormones (e.g., parathyroid hormone, 1,25-dihydroxyvitamin D₃, calcitomin, and glucorticoids) and locally acting cytokines, which are factors controlling bone remodeling [1]. Whether this mechanism for bone remodeling coincides with the cow described in this report needs to be elucidated further.

Although not common, osseous metaplasia can develop following either metastatic calcification from systemic disease or dystrophic calcification from local tissue damage [29]. The current bovine case was simultaneously affected with chronic sepsis. Even if the latter was related to the development of cholangiocarcinoma that could have had an adverse effect on immunity, apparently, metastatic calcification associated with systemic bacterial infection was unlikely the case with osseous metaplasia in the present cow, because osseous metaplasia occurred exclusively in one of the mesenteric lymph nodes, whereas other organs and tissues, with or without metastatic cholangiocarcinoma, were not accompanied by areas of mineral deposition. On the other hand, the observation that necrotic tissue within the cancer (cholangiocarcinoma), primary or metastatic, showed no mineralized areas allowed dystrophic calcification to be excluded.

Osseous metaplasia implies differentiation of local cells at nonosseous tissues into osteogenic cells as a response to a variety of pathologic states, including those that are attributed to traumatic, neoplastic, ischemic, necrotic, inflammatory, or metabolic insults [5, 10, 29]. The histogenesis of osseous metaplasia in cancer is a subject of debate. It has been suggested that in ovarian cystadenoma or adenocarcinoma, a metaplastic process involving multipotential stromal stem cells results in bone formation [2, 3]. In addition, osteocartilaginous metaplasia in a case of laryngeal squamous cell carcinoma has been viewed as a change determined by the microenvironment of the tumor-host interface [12]. In the present bovine case, the mesenteric lymph node displaying osseous metaplasia not only demonstrated macrophage/giant cell-reaction to necrotic areas of metastatic cholangiocarcinoma but also represented conspicuous fibroblastic and capillary proliferation in the connective tissue stroma, most likely indicating high activity of stromal mesenchymal cells resident at this lymph node. The present study failed to identify reparative response by phagocytes—macrophages and/or giant cells—around necrotic areas of primary cholangiocarcinoma or at its metastatic sites other than the mesenteric lymph node undergoing osseous metaplasia. This evidence supported the notion that the bone trabeculae probably were derived from activated stromal fibroblasts capable of differentiating to osteoblasts, but not from neoplastic cells themselves [1, 8, 10, 13]. Taken together, the
biologic mechanism of osseous metaplasia was likely caused by stromal activation that was provoked by a reaction to tissue damage and repair following metastasis of cholangiocarcinoma to the mesenteric lymph node. Studies have strongly suggested that differentiation of fibroblasts to osteoblasts is induced by bone morphogenetic proteins (BMPs) which are members of transforming growth factor-β (TGF-β) [13, 30, 31].

The incidence of osseous metaplasia is uncommon in lymph nodes. Albeit less severely, lymphoplasmacytic and eosinophil infiltrates were present in the connective tissue stroma around metaplastic bone trabeculae in the mesenteric lymph node, so pre-existing lymphadenitis cannot be rigidly ruled out from predisposing factors that account for a localized change in microenvironment resulting in osseous metaplasia. In recent years, cutaneous osseous metaplasia in dogs with hyperadrenocorticism and calcinosis cutis is considered due to local production of specific growth factors such as TGF-β by inflammatory cells in the dermis [1]. In Castleman’s disease, a benign lymphoproliferative disorder, an axillary lymph node revealed lymphadenopathy with osseous metaplasia for which interleukin (IL)-6 may be responsible [30]. However, it could not be confirmed whether specific growth factors certainly played a role in the development of osseous metaplasia in the mesenteric lymph node of the present cow.

**Conclusion**

To the best of the author’s knowledge, this study appears to be the first to demonstrate the osseous metaplasia in the lymph node with metastatic cancer. A precise explanation for this phenomenon remains to be determined, though this bovine case may be useful in the future when researching human cancer associated with osseous metaplasia.

**Acknowledgment**

I wish to thank the veterinary meat inspectors of the Hokkaido Meat Inspection Center for collecting the material and for follow-up collaborations.

**References**


