Re-evaluation of Metaplastic Osteogenesis in the Bovine Lymph Node with Metastatic Cholangiocarcinoma: A Supplemental Report

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Abstract

Introduction: For deepening knowledge of the developmental process involving metaplastic osteogenesis in extraskeletal tumor, histopathological re-evaluation was conducted on osseous metaplasia in the bovine lymph node with metastatic cholangiocarcinoma, which was previously reported in this journal by the author.

Presentation of Case: In an aged Holstein-Friesian dairy cow that exhibited intrahepatic cholangiocarcinoma with widespread metastasis, a mesenteric lymph node with metastatic cancer had multifocal areas of osseous metaplasia. Abundant stromal connective tissue of the metastatic cancer showed prominent proliferation of capillaries and mesenchymal cells. There were no definite transitional features between mesenchymal cells associated with bone tissue and cancer cells. A variety of stages toward differentiating into osseous component included occurrence of (1) focal areas of dense collagen fibers accompanied by many mesenchymal cells, (2) osteoid surrounded by a single to multilayer of osteoblasts, (3) trabeculae of woven bone containing many osteocytes, and (4) bone resorption associated with osteoclastic activity in more differentiated bone trabeculae. These findings suggested that osseous metaplasia might have developed through the process identical to intramembranous ossification, as seen in flat bone formation in the developing fetus. Furthermore, this metaplastic osteogenesis was associated with the process involving the coordinated activities of osteoblasts and osteoclasts, as seen in remodeling of bone tissue throughout postnatal life.

Conclusion: Although metaplastic bone did not fully differentiated into mature bone, the pathway of metaplastic osteogenesis might have followed the developmental stages identical, to some degree, to the process that controls skeletal maturation and remodeling.

Keywords: Bovine; lymph node; metaplastic osteogenesis; metastatic cancer

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Introduction

Although uncommon, occurrence of osseous metaplasia in a variety of benign or malignant tumor has been documented in human beings [1-17] and domestic or other animals [18-24]; most of reported cases being sporadic and isolated. To the author’s knowledge, it seems that detailed developmental process of such osteogenesis has been not fully been understood. This is also true for the precise conditions and biological signals for spontaneous heterotopic bone formation [25]. In this journal, the author has previously reported a bovine case of spontaneous osseous metaplasia in a mesenteric lymph node with metastasis from cholangiocarcinoma [26]. In that case, high activity of mesenchymal cells resident at the lymph node was suggested to play a role in metaplastic osteogenesis, but no further details were unavailable. This limitation and the opportunity for investigation prompted to define more exactly the pathology of such a metaplastic condition. The present supplemental report describes the developmental process of metaplastic bone tissue in this bovine lymph node. This is a re-evaluation undertaken from the histopathological viewpoint.

Case Presentation

This case study concerns a Holstein-Friesian dairy cow, aged 8 years and 9 months, which, on postmortem examination, exhibited multiple tumor nodules in many body sites. Such tumor lesions were histopathologically and immunohistochemically diagnosed as intrahepatic cholangiocarcinoma and its metastasis to many thoracic and abdominal organs and tissues. Their detailed gross and histopathological findings have been described elsewhere [26]. The present investigation focused on a mesenteric lymph node that underwent metastasis from the cancer.

Fig. 1. In the mesenteric lymph node with cholangiocarcinoma metastasis (asterisks), there is a peninsular form of woven bone trabeculae that are partly mineralized. HE. Bar = 180 μm.

Fig. 2. Mesenteric lymph node represents multiple, insular, woven bone tissues with mineralization, which are formed in abundant stromal connective tissue of metastatic cholangiocarcinoma (asterisk). HE. Bar = 240 μm.
Tissue samples for histopathological re-examination were taken anew from many portions of the affected mesenteric lymph node. Histopathological examination of formalin-fixed, paraffin-embedded, hematoxylin and eosin (HE)-stained sections revealed that the lymph node was almost completely replaced by desmoplastic cancer lesions that metastasized from primary cholangiocarcinoma. Within these cancer lesions there were frequent necrotic foci, which were not associated with mineralization. Multifocal areas of bone tissue were present in the stromal connective tissue of the cancer. Stromal connective tissue contained large numbers of mesenchymal cells and many capillaries. In general, a background of slightly mucinous stroma was present. Bone tissue mingled irregularly with cancer nests. Bone tissue had a variety of distribution with a haphazard arrangement, such that in some regions, there was peninsular proliferation (Figure 1), and in other regions, there was insular proliferation (Figure 2). Although lesions of osseous metaplasia showed a close topographical relationship to those of cancer as such, there were no definite transitional features between cancer cells and osteoblasts or mesenchymal cells associated with bone tissue.

Fig. 3. In the stromal connective tissue of cholangiocarcinoma metastasizing to the mesenteric lymph node, fibrillary matrix (asterisks) of collagen fiber deposition with mesenchymal cells is continuous with small area of osteoid. HE. Bar = 30 µm.

Fig. 4. A double layer of osteoblasts surrounds thin osteoid in which shadow nuclei (arrows) suggestive of dying osteocytes are seen. HE. Bar = 40 µm.

Fig. 5. Osteoid surrounded by a single layer of osteoblasts shows partly mineralized areas that are associated with osteocytes with pyknotic nuclei (arrows). HE. Bar = 40 µm.

Fig. 6. A woven bone trabecula adjacent to lesions of metastatic cholangiocarcinoma (asterisk) exhibits more differentiated areas that are undergoing osteoclastic resorption (arrows). HE. Bar = 120 µm. Inset: Higher magnification of Fig. 6 shows features of Howship’s lacunae (arrows) associated with osteoclasts. HE. Bar = 60 µm.
Lesions of osseous metaplasia exhibited a variety of stages toward differentiating into osseous components. First, there were focal areas of dense collagen fibers that formed fibrillary matrix. These areas were accompanied by abundant mesenchymal cells that occasionally exhibited mitotic figures and had hyperchromatic nuclei and basophilic cytoplasm. Many of these fibrous areas showed features transitional to osteoid, which contained osteocytes with chromatin-rich nuclei similar to those of mesenchymal cells (Figure 3). Second, a single to double layer of osteoblasts covered the surface of osteoid, which contained shadow nuclei lacking nuclear detail, probably indicative of dying osteocytes (Figure 4). Features of osteoblasts resembled those of mesenchymal cells. Third, many of large-sized osteoid tissues represented covering by a single layer of osteoblasts, and matrix of osteoid represented varying degrees of mineralization (Figure 5). Some osteocytes showed pyknotic nuclei within partly mineralized osteoid matrix. Last, there was formation of woven bone trabeculae, which indicated immature form of bone tissue and containing many osteocytes. More differentiated portions of trabeculae of woven bone, where mature bone matrix became more prevalent than numbers of osteocytes, underwent osteoclastic resorption (Figure 6). Occasionally, those areas, which were associated with osteoclasts, showed formation of concave surfaces identical to Howship’s lacunae (Figure 6, inset). There was no feature indicating replacement of woven bone by lamellar bone, nor was the presence of cementing lines and osteons.

Discussion

Although there are relatively many reports on the occurrence of osseous metaplasia in a variety of benign or malignant tumor of human beings and animals [1-24], it appears that there are few descriptions targeted specifically to the developmental process involving metaplastic osteogenesis in extraskeletal tumors. This supplemental report concerned a bovine case of osseous metaplasia in a mesenteric lymph node that underwent metastasis from intrahepatic cholangiocarcinoma. Cholangiocarcinoma has been reported with a relatively high frequency in human beings [27-30] and many domestic animal species [31-35], but this type of cancer is most unlikely to be bone-forming tumor. Necrotic foci within metastatic lesions of cholangiocarcinoma were not associated with mineralization (calcification), probably excluding the likelihood that dystrophic mineralization capable of differentiating into ectopic ossification [36] was implicated in the osseous metaplasia. No transitional features between cancer cells and osteoblasts could be seen elsewhere. Thus, it is unlikely that the cell of origin of bone formation in the lymph node was metaplastic or dedifferentiated cancer cells. Moreover, osseous metaplasia occurred exclusively in a mesenteric lymph node and not in other organs and tissues with metastatic cholangiocarcinoma. Based on these evidences, it is thought that such metaplastic osteogenesis might have been attributed solely to a localized change in microenvironment of the lymph node, as alluded to previously [26]. Transformation of mesenchymal cells to osteoblasts, which could have occurred within stromal connective tissue of metastatic cholangiocarcinoma in the lymph node, was a change that developed in microenvironment conceptually accounted for by the tumor-host interface [37-39], although factor(s) initiating mesenchymal cell’s transformation could not be determined.

Heterotopic bone, which is due possibly to metaplasia [36], can form at four kinds of sites, including nonskeletal soft tissues, nonosseous musculoskeletal tissues, entheses, and paraskeletal tissues [25]. Metaplastic bone usually arises in ligaments, tendons, joint capsules, organ capsules, fasciae, cartilage tissue, and stromal tissue of muscle organs and glands [25]. Thus, incidence of osseous metaplasia in lymph nodes may be what the exception rather than the rule.
The exact pathogenesis of ossification in tumors is unclear. In renal cell carcinoma, it has been hypothesized that bone tissues originate through either the dedifferentiation of neoplastic cells into a sarcomatous proliferation, or the production of a dense collagenous matrix by mesenchymal cells with subsequent mineralization and organization into bone [10, 16, 17]. The second category was different strictly from, although conceptually similar to, the current bovine case, where the stromal connective tissue itself showed no mineralized changes preceding the appearance of bone tissue. Occurrence of areas of dense collagen-fiber deposition was associated with large numbers of mesenchymal cells but not associated with mineralization. There was formation of pre-mineralized osteoid and woven (immature) bone with a single layer of osteoblasts lined up along their surface, as well as the appearance of dying osteocytes with the progression of mineralization in osteoid matrix. Thus, it is likely that osseous metaplasia begun from primary proliferations of mesenchymal cells, followed by osteoblastic differentiation of the cells. Such pattern of development is thought to be identical to the process of intramembranous bone formation, as seen in flat bones of the skull in the developing fetus and during postnatal life [36, 40, 41]. Furthermore, the surface of more differentiated areas of bone trabeculae exhibited covering by osteoblasts and osteoclasts, and osteoclastic resorption formed concave surfaces consistent with Howship’s lacunae. It is considered that metaplastic osteogenesis was associated with the process involving the coordinated activities of osteoblasts and osteoclasts, as seen in remodeling of bone tissue throughout postnatal life [36, 40, 41]. Among a variety of systemic hormones and local factors that control bone remodeling, bone morphogenetic proteins (BMPs) seem to be most important in provoking osseous metaplasia since they not only stimulate osteoblast differentiation but also induce heterotopic bone formation in vivo [36]. BMPs are considered as possible therapeutic option in diseases for which enhancing bone formation is desirable [36, 41, 42].

Based on the results from the case under study, it appeared that metaplastic bone did not fully differentiated into mature lamellar bone like the adult skeleton. Characteristically, metaplastic osseous tissues represented a variety of differential stages, lacking specific unity each other about the developmental sequence. However, metaplastic bone did not necessarily represent morphological processes different entirely from those seen in bones of the skull in the developing fetus and during postnatal life.

**Conclusion**

It is suggested that pathway of metaplastic osteogenesis might have followed the developmental stages identical, to some degree, to the process that controls skeletal maturation and remodeling. Histopathological findings from this bovine case raise a possibility that they may provide an animal model to help elucidate the developmental process of osseous metaplasia in human cancer.

**References**

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