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Original contribution

Vascular patterning in human heterotopic ossification ☆

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Summary Heterotopic ossification (HO, also termed myositis ossificans) is the formation of extra-skeletal bone in muscle and soft tissues. HO is a tissue repair process gone awry, and is a common complication of surgery and traumatic injury. Medical strategies to prevent and treat HO fall well short of addressing the clinical need. Better characterization of the tissues supporting HO is critical to identifying therapies directed against this common and sometimes devastating condition. The physiologic processes of osteogenesis and angiogenesis are highly coupled and interdependent. However, few efforts have been made to document the vascular patterning within heterotopic ossification. Here, surgical pathology case files of 29 human HO specimens were examined by vascular histomorphometric analysis. Results demonstrate a temporospatial patterning of HO vascularity that depends on the "maturity" of the bony lesion. In sum, human HO demonstrates a time- and space-dependent pattern of vascularization suggesting a coupled pathophysiologic process involving the coordinate processes of osteogenesis and angiogenesis. Further imaging studies may be used to further characterize vasculogenesis within HO and whether anti-angiogenic therapies are a conceivable future therapy for this common condition.

1. Introduction

Heterotopic ossification (HO, also termed myositis ossificans) is the pathologic development of extra-skeletal bone, which develops after musculoskeletal trauma, spinal cord injury, or severe burns. In a distinct but related genetic disease, patients with an activating mutation in the type I bone morphogenetic protein (BMP) receptor ACVR1 develop ectopic bone after minimal soft tissue trauma [1]. HO is surprisingly common, and has been reported to occur at a frequency of 90% after certain types of hip arthroplasty or acetabular fractures [2-7]. HO represents a major health burden to the patient, associated with pain, mobility impairments, deep vein thrombosis, nerve entrapment, and poor wound healing. Reports of disability associated with HO are variable, but may exceed a frequency of 20%, owing to soft tissue loss, joint contracture, and chronic pain [8].

The histopathologic appearance of heterotopic ossification evolves over time. Early lesions demonstrate a hypercellular proliferation of spindle cells often with little bone matrix. Spindled areas often have features of nodular fasciitis (NF), including high numbers of normal mitotic figures, scattered multinucleated giant cells, and extravasated erythrocytes. As ossification ensues, woven bone with prominent osteoblast lining is characteristic. Cartilage and endochondral ossification

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may be seen, but are usually focal and not seen in most cases. More mature lesions show thickened trabeculae of lamellar bone with less prominent osteoblastic rimming. Late in the evolution of HO, lesions develop an appearance similar to compact bone. On intact surgical pathology specimens, a zonation phenomenon is often appreciated, with the most mature bone tissue observed at the periphery. On resection, HO is most often bounded by a thick fibrous capsule. Despite the well-characterized phasic appearance of HO that forms a temporospatial continuum, a detailed vascular histomorphometric analysis is lacking. Here, we report on the vascular patterning of human HO with a particular emphasis on vascular modeling across the histologic spectrum of HO.

2. Materials and methods

2.1. Identification of HO samples

Thirty-five cases of HO were identified in our surgical pathology archives (Johns Hopkins University and University of California, Los Angeles). Cases were obtained under IRB approval with waiver of informed consent. All material was coded so as to protect the confidentiality of personal health information. Six cases were excluded due to insufficient material to analyze. A total of 29 cases were included in the study, with 2 independent pathologists verifying the diagnostic accuracy (E.M., A.W.J.).

2.2. Image acquisition and categorization of HO samples

Random histologic images were taken of each sample with the goal of encompassing distinct areas of each lesion. Depending on the size of the specimen, between 3 and 17 images of each case were taken. A total of 177 images were analyzed. Next, images were examined (by M.C. and A.W.J) and categorized base on their predominant histologic appearance. The following five subcategories were employed:

(1) Nodular fasciitis (NF)–like – sheets and fascicles of plump spindled myofibroblastic cells with minimal bone formation;
(2) HO with woven bone – composed predominantly of woven bone with prominent osteoblastic rimming;
(3) HO with lamellar bone – composed predominantly of thickened trabeculae of lamellar bone;
(4) Fibrous capsule – the thickened fibrous capsule immediately peripheral to lesional tissue;
(5) Cartilaginous areas – areas of prominent cartilaginous differentiation or endochondral ossification.

2.3. Vascular histomorphometric analysis of HO samples

Each image was analyzed in a blinded fashion to determine the following parameters: blood vessel number (as defined by the total vessel number per 10× field), blood vessel area (as defined by the total vascular area per 10× field), blood vessel density (as defined as blood vessel area/tissue area per 10× field), blood vessel size (as defined by blood vessel area/number per 10× field), and blood vessel wall thickness (as defined by mean vessel wall thickness per 10× field). Vessel number was counted manually, while all other calculations were performed using Adobe Photoshop CS6 (San Jose, CA). Vessel area was determined using the magic wand tool in Adobe Photoshop in conjunction with the measurement log function to measure area in pixel units. Density was calculated by dividing vessel area by total photograph tissue area, as determined using Adobe Photoshop. To account for the difference between a large number of small vessels and a small number of large vessels, vessel area was divided by vessel number to determine average vessel size in each slide. Vessel thickness was measured using the "ruler tool" in Adobe Photoshop, and thickness was measured in pixel units. Use of Adobe Photoshop– based quantifications is illustrated in the Supplementary Figure. Graphical representations were constructed using GraphPad Prism (La Jolla, CA).

3. Results

3.1. Patient demographics and histologic appearance

A total of 29 cases of HO were included in our study. Patients were evenly distributed by age (14 male, 15 female). Mean patient age was 37.9 years (range, 6–76) at the time of resection. HO affected the lower extremity in most cases (n = 17), followed by the upper extremity (n = 7), or back/paraspinal area (n = 3). Many cases were juxta-articular, including the hip (n = 4), knee (n = 5), elbow (n = 1), wrist or ankle (n = 3). Patient demographics and specific location of each lesion are further detailed in the Supplementary Table.

Typical histologic features of each HO were next examined, using our previously described categories to describe the histologic spectrum of HO (Fig. 1). Categories included:

(1) NF-like areas, in which sheets and fascicles of spindled fibroblastic/myofibroblastic cells were observed resembling nodular fasciitis or granulation tissue (Fig. 1A and B). Only scattered/minimal bone formation was observed in these areas, which tended to involve the central aspects of the lesion. Blood vessels in NF-like areas consisted of numerous, slender, elongated, and thin-walled vascular channels with a similar orientation to the spindled stroma. Samples with NF-like areas were less common (n = 11 cases).
(2) HO with woven bone, in which the lesional bone is predominantly composed of woven bone with prominent osteoblastic rimming (Fig. 1C and D). Blood vessels within the interstices between woven bone trabeculae were thin walled, slightly dilated, and numerous. All cases showed at least some areas with woven bone.