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Origin and Therapeutic Strategies for Ectopic Bone Formation in Skeletal Muscle

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Abstract

Heterotopic ossification (HO) is caused by trauma, neurogenic insults, and genetic disorders. Recent detailed analyses have revealed a cellular origin for ectopic bone formation as novel mesenchymal progenitors. The differentiation of these into osteogenic lineages is induced by a pathological microenvironment in soft tissues outside the skeletal tissue, which includes inflammation. Multiple therapeutic strategies for preventing ectopic bone formation have recently emerged, including inhibition of bone morphogenetic protein signaling and retinoid signaling.

Keywords: Bone morphogenetic protein; Ectopic bone; Fibrodysplasia ossificans progressiva; Heterotopic ossification; Mesenchymal progenitor

Introduction

Heterotopic ossification (HO) is characterized as the formation of ectopic bone in soft tissues outside the skeletal tissue. There are three etiologies of HO, namely trauma, neurogenic or surgical insults, and genetic disorders [1]. HO is most commonly observed after surgical trauma such as total hip arthroplasty (THA) [2], although other affected sites include the elbow, knee, shoulder, and ankle [1]. Damage to the central nervous system such as spinal and head injuries is responsible for neurogenic HO, with the hip joint being the most common site of neurogenic ectopic bone formation.

HO can also be seen in genetic disorders such as Fibrodysplasia Ossificans Progressiva (FOP), Albright's Hereditary Osteodystrophy (AHO), and Progressive Osseous Heteroplasia (POH) [3-7]. FOP is a debilitating disorder characterized by progressive HO, which has congenital malformations of the big toes as an early hallmark [4]. Classic FOP is caused by an activating mutation (617G > A; R206H) in the *Alk2/ACVR1* gene encoding activin receptor-like kinase 2, a bone morphogenetic protein (BMP) type I receptor [5]. Atypical FOP patients carry various heterozygous missense mutations of *Alk2/ACVR1* [5]. Although heritable FOP shows transmission in an autosomal dominant manner, most cases of FOP are sporadic. In addition to FOP, AHO and POH, ectopic calcification in the skeletal muscle is also seen in *mdx* mice, a dystrophin-deficient animal model of Duchenne muscular dystrophy [8].

Cellular Origin of Ectopic Bone in Soft Tissues

The pathophysiology of HO is not well understood. One theory is that HO results from the differentiation of osteogenic progenitors that are pathologically induced by local and systemic factors [1]. HO results from excessive BMP signaling in the inflammatory environment, which, together with increased expression of other osteogenic cytokines, can be triggered by injury to the soft tissue. Direct evidence for the involvement of enhanced BMP signaling in HO was shown by the discovery of a genetic mutation in the *Alk2* gene that causes FOP. Mutant *Alk2* (R206H in classic FOP) is a constitutively active form of the BMP receptor, therefore BMP signaling is active even in the absence of BMP and increased expression of BMP augments this signaling further [4,5]. In addition to augmented BMP signaling, an inflammatory milieu is also required for the development of ectopic ossification [9]. As traumatic and surgical injuries cause HO, this suggests that an inflammatory environment is important in ectopic bone formation.

The cellular origin of osteogenic progenitors is not well characterized. However, recent sophisticated analyses have clarified the progenitors for ectopic bone in soft tissues. Platelet-derived growth factor receptor (PDGFR)α$^+$ mesenchymal progenitor cells, distinct from satellite cells, have been shown to be located in mouse and human interstitial spaces within skeletal muscle [10,11]. These cells are the sources for fat infiltration and fibrosis of skeletal muscle [10,12] and undergo osteogenic differentiation in response to BMP stimuli and/or osteogenic conditions [10].

Interestingly, a lineage-tracing study revealed that vascular smooth muscle cells were not involved in any stage of HO [13]. Satellite cells, which are myogenic stem cells and MyoD-expressing myogenic precursors, show osteogenic responses in culture; however, they play a minimal role in the development of HO [11,13]. By contrast, cells that expressed Tie2 contributed greatly to osteogenic, chondrogenic, and fibroproliferative stages in the heterotropic endochondral anlagen [13]. Tie2-expressing progenitor cells respond to inflammatory triggers, differentiate into osteogenic lineages, and thereby contribute to HO [13]. Moreover, recent investigations revealed that Tie2$^+$PDGFRα$^+$Sca-1$^+$ progenitors in skeletal muscle interstitium are involved in HO [14]. FACS-isolated PDGFRα$^+$ progenitors showed osteogenic differentiation both *in vitro* and *in vivo* [11], and have been observed surrounding ectopic bone tissues after neurogenic trauma in human HO samples. This supports the notion that PDGFRα$^+$ mesenchymal progenitors are the origin of ectopic bone formation [11].

One previous report documented the conversion of vascular

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endothelial cells into multipotent stem-like cells in an Alk2 receptor-dependent manner, followed by differentiation into osteoblasts, chondrocytes, and adipocytes [15]. However, this study used Tie2 as an endothelial marker. Although this is a good marker for endothelial lineages, it is also expressed in other progenitors [14]. Therefore, it is likely that $Tie2^+PDGFR\alpha^+Sca\text{-}1^+$ progenitors in interstitium, but not endothelial cells, are responsible for ectopic bone formation *in vivo* [11,14]. As Sca-1 is expressed in other cell types, including endothelial cells, and there is no human counterpart, $PDGFR\alpha^+$ mesenchymal progenitors in skeletal muscle are likely to be one of the best cell sources for the major origin of HO both in mouse and human [11].

Prevention and Therapeutic Strategies for Heterotopic Ossification

Two common prophylactic therapies for HO are nonsteroidal anti-inflammatory drugs (NSAIDs) and radiation therapy. NSAIDs, such as indomethacin, are effective in the prevention of HO if treatment is started early. However, prolonged use of NSAIDs is sometimes associated with adverse drug reactions such as gastritis and ulcers, and bone nonunion after fracture is also reported [1]. Preoperative radiation is effective in the prevention of HO after total hip or knee arthroplasty and it is hypothesized that osteogenic progenitors in skeletal muscle are radiosensitive in the early phase of HO development [1,2]. Combined postoperative radiotherapy and indomethacin are reported to be effective at preventing HO after total hip replacement [16].

With regard to therapy for genetic abnormal ectopic bone formation, progress has particularly been made in FOP. As indicated above, a constitutively active *Alk2* mutation is responsible for FOP etiology [4,9]. Inflammation is believed to cause tissue damage and the activation of mesenchymal progenitors that differentiate to form a second skeleton of HO under BMP signaling. Therefore, the surgical removal of ectopic bone is not only ineffective for the treatment of FOP but may also lead to the formation of additional ectopic bone. A selective inhibitor for BMP type I receptor kinase, LDN-193189, was proven to be effective at inhibiting BMP signaling and reducing ectopic ossification and functional impairment in mice expressing a highly constitutively active *Alk2* mutation (Q207D). Corticosteroid treatment is also effective in this mouse model, indicating the importance of suppressing BMP signaling and inflammation for the treatment of FOP [9].

Retinoid signaling is a strong inhibitor of chondrogenesis that can block heterotopic endochondral ossification [17]. Prechondrogenic and chondrogenic stages of HO are highly sensitive to retinoid acid receptor-γ (RARγ), and the RARγ agonists NRX204647 and CD1530 are effective at blocking BMP-induced, inflammation-induced, and *Alk2* mutation-induced HO in animal models [17]. RARγ agonists inhibit BMP signaling by promoting the degradation of BMP-regulated Smads in a proteosome degradation pathway. They are also effective at blocking HO in the chondrogenic phase of bone formation. Since all types of HO require cartilaginous scaffolds for bone formation, RARγ agonists could be a novel therapeutic option for the treatment of ectopic bone.

The activation of *Alk2* leads to the upregulation of the transcription factor called inhibitor of differentiation-1 (Id1). Screening of more than 1000 FDA-approved compounds for the inhibition of Id1 promoter activity by mutant *Alk2* in FOP recently identified anti-anginal agents as potential candidates [18]. Treatment with one of these potential drug candidates, perhexiline maleate, a mild blocker of L-type calcium channel and a prophylactic antianginal drug, resulted in a 38% reduction of HO volume in a BMP-implanted mouse model. Therefore, perhexiline and its derivatives could be used as clinically applicable drugs for HO in FOP and related disorders.

Several miRNAs are known to be upregulated during osteogenic differentiation. OstemiRs include members of the miR-541 and miR-30 families that regulate osteoblastic and osteocytic differentiation from mesenchymal stem cells in a stage-specific manner [19]. Knockdown experiments showed that miR-541 is a negative regulator of osteoblastic differentiation. By contrast, inhibition of miR-146b-5p and miR-424, which are upregulated during osteogenic differentiation from $PDGFR\alpha^+$ mesenchymal progenitors, resulted in the suppression of osteocyte maturation, suggesting a positive role for these miRNA in osteogenesis [11]. The targeting of miRNAs involved in either the promotion or inhibition of osteogenesis is one of the novel therapeutic approaches for the treatment of ectopic bone in soft tissues [11,19]. These promising therapeutic strategies could be applicable not only to FOP, but also to other conditions involving ectopic bone formation in soft tissues.

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