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Role of Altered Signal Transduction in Heterotopic Ossification and Fibrodysplasia Ossificans Progressiva

Eileen M. Shore and
Department of Orthopaedic Surgery, University of Pennsylvania School of Medicine, 426 Stemmler Hall, 3450 Hamilton Walk, Philadelphia, PA 19104-6081, USA. Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. The Center for Research in FOP and Related Disorders, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Frederick S. Kaplan
Department of Orthopaedic Surgery, University of Pennsylvania School of Medicine, 426 Stemmler Hall, 3450 Hamilton Walk, Philadelphia, PA 19104-6081, USA. Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. The Center for Research in FOP and Related Disorders, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. Department of Orthopaedic Surgery, Hospital of the University of Pennsylvania, Silverstein Pavilion - Second Floor, 3400 Spruce Street, Philadelphia, PA 19104, USA

Eileen M. Shore: shore@mail.med.upenn.edu; Frederick S. Kaplan: frederick.kaplan@uphs.upenn.edu

Abstract

Heterotopic ossification is a pathologic condition in which bone tissue is formed outside of the skeleton, within soft tissues of the body. The extraskeletal bone that forms in these disorders is normal; the cellular mechanisms that direct cell fate decisions are dysregulated. Patients with fibrodysplasia ossificans progressiva (FOP), a rare human genetic disorder of extensive and progressive heterotopic ossification, have malformations of normal skeletal elements, identifying the causative gene mutation and its relevant signaling pathways as key regulators of skeletal development and of cell fate decisions by adult stem cells. The discovery that mildly activating mutations in ACVR1/ALK2, a bone morphogenetic protein (BMP) type I receptor, is the cause of FOP has provided opportunities to identify previously unknown functions for this receptor and for BMP signaling and to develop new diagnostic and therapeutic strategies for FOP and other more common forms of heterotopic ossification, as well as tissue engineering applications.

Keywords

Activin A type I receptor; ACVR1; Activin-like kinase 2; ALK2; Bone morphogenetic proteins; BMP; BMP receptors; BMP signaling; Endochondral ossification; Fibrodysplasia ossificans progressiva; FOP; Heterotopic ossification; Extraskeletal bone formation

Correspondence to: Eileen M. Shore, shore@mail.med.upenn.edu.

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Introduction

Bone formation is normally limited to the skeletal system. Skeletal elements form during embryonic development and then, throughout life, undergo a balanced process of bone remodeling (bone formation and resorption) to maintain bone structure and strength. In response to injury, bone healing occurs through a well-described process of repair that involves an inflammatory inductive stage and recruitment of osteoprogenitor cells that participate in new bone formation [1].

Alterations in the usual processes that regulate the timing and location of bone formation can cause disease. Heterotopic ossification (HO) is a pathologic condition in which bone forms outside of the normal skeleton in nonskeletal tissues [2, 3]. During HO, bone tissue forms through endochondral or intramembranous processes and produces qualitatively normal bone tissue. In this condition, the induction of the bone formation process is abnormally regulated.

In most cases, HO is not a primary pathologic event, but occurs secondary to other clinical conditions. These acquired, or nonhereditary, forms of heterotopic bone formation in connective tissues are most frequently associated with severe soft tissue trauma, and are a common complication of central nervous system injury (eg, traumatic brain injuries, spinal cord lesions, tumors, encephalitis), total hip arthroplasty, deep tissue burns, multiple forms of trauma (including war-related injuries that lead to amputations), and end-stage cardiac valve disease [2–7]. HO has significant consequences for the health of affected individuals, causing restricted joint range of motion, severe pain, and limitations to prosthetic use.

As initially suggested by Chalmers et al. [8], at least three components are necessary for the de novo formation of ectopic bone: an induction event that initiates molecular and cellular signals that lead to bone formation; progenitor cells that are recruited to form bone and/or cartilage; and a permissive tissue microenvironment that supports the progression to bone tissue. However, because the onset of HO cannot be easily predicted, investigations to examine the inductive events that lead to HO have been limited. Studies have investigated gene expression and signaling pathways that are candidates for inducing and promoting heterotopic bone formation, but no definitive mechanisms have been identified through these approaches.

In addition to the acquired forms of HO, rare inherited disorders of extensive and progressive HO have been identified: progressive osseous heteroplasia (POH) and fibrodysplasia ossificans progressiva (FOP) [9•]. In both of these clinically distinct disorders, HO typically begins during childhood, with progressive and episodic bone formation that continues throughout life. Both FOP and POH are caused by single gene mutations. Discovery of these disease-causing genes identified them as critical components of the regulatory mechanisms that direct cell fate decisions and bone tissue formation and provide a focus for understanding the process of HO. In this article, we will focus on the altered signaling pathway that is caused by mutations in FOP.

Fibrodysplasia Ossificans Progressiva

FOP (MIM 135100) is a rare human genetic disease of distinct skeletal malformations and progressive, episodic heterotopic endochondral ossification that occurs at a population frequency of about one per 2 million. In FOP, bone forms at extraskeletal (heterotopic) sites within connective tissues such as skeletal muscle, tendon, ligament, fascia, and aponeuroses; smooth muscle and cardiac muscle are spared [10, 11]. FOP can be inherited through an autosomal-dominant pattern; however, due to the severe debilitating nature of this condition,

genetic transmission is observed in only a small number of families and most known cases occur in families with no prior history of FOP [12].

The heterotopic bone that forms in FOP is normal skeletal bone by most criteria including histology, biochemistry, metabolism, radiology, and biomechanics [10, 11]. The mature ectopic bone can form bone marrow cavities, undergo bone remodeling, and respond to fracture with a normal repair process [10, 11]. The consequence is a skeletal-like bone organ system that develops in the soft tissues of FOP patients in response to the mutated ACVR1 bone morphogenetic protein (BMP) type I receptor.

In patients with FOP, HO begins in soft tissues after birth, with the first event typically occurring in children before 5 years of age [10, 13]. In addition, specific skeletal malformations also occur in people with FOP, indicating that the underlying gene mutation functions during normal embryonic skeletal development as well as in regulating the cell fate decisions of progenitor cells in nonembryonic tissues [10, 13]. Congenital malformation of the great toes provides the earliest evidence of FOP and is an invariant feature of patients with a classic clinical presentation of the disease. Toe malformation is the most recognizable skeletal feature of FOP, although other skeletal changes are also commonly found such as thumb malformations, clinodactyly, short broad femoral necks, spine/vertebral malformations, and widely distributed osteochondromas [10, 13, 14•].

Among cases of heterotopic endochondral ossification, patients with clinical features that are not commonly associated with FOP have also been identified [14•]. Patients who have characteristics that are uncommon in FOP patients, along with the classic defining FOP features of progressive HO and great toe malformations, are described as having “FOP-plus.” Patients who present with significant deviation from the standard clinical presentation of one or both of the two classic defining features of FOP, most commonly milder or more severe skeletal malformation of first digits, are described as having “FOP variants.”

Genetic Mutations in FOP

Genetic linkage analysis to chromosome 2q23–24 and positional cloning led to the identification of *ACVR1/ALK2* as the mutated gene in FOP [15••]. ACVR1 (Activin A type I receptor; also known as ALK2, activin-like kinase 2) is a type I receptor for BMPs. BMPs were identified as inducers of osteogenic differentiation [16], and a series of studies had determined that BMP signaling is altered in cells from FOP patients [17–20].

All individuals with the classic features of FOP (progressive HO and great toe malformations) contain the same heterozygous single nucleotide substitution that changes amino acid 206 from arginine to histidine (c.617G>A; R206H). The mutated amino acid occurs within the glycine-serine (GS) region of the cytoplasmic domain of ACVR1/ALK2 and is evolutionarily highly conserved.

All patients with atypical FOP who have been examined also have heterozygous *ACVR1* missense mutations in highly conserved amino acids [14•]. The c.617G>A; R206H mutation has been found in most FOP-plus patients, suggesting that the atypical associated features are either unrelated to *ACVR1* mutation or extremely rare manifestations of the mutation. Novel non-R206H *ACVR1* mutations in the GS or protein kinase domains of *ACVR1* occur in two cases of FOP-plus and in all FOP variants.

Effects of FOP ACVR1 Mutation on BMP Signaling

The BMP Signaling Pathway

Signal transduction through the BMP pathway is mediated through heterotetramer receptor complexes of two type I and two type II serine-threonine kinase receptors. In response to ligand binding, type II receptors activate signaling through phosphorylation of the cytoplasmic GS domain of the type I receptors [21–23]. The activated type I receptors mediate downstream signaling through BMP pathway-specific Smad proteins and through mitogen-activated protein kinase pathways [21, 24]. In addition to ACVR1/ALK2, the BMP type I receptor mutated in FOP, BMP signal transduction is mediated through the BMPR1A/ALK3 and BMPRIB/ALK6 type I receptors.

BMPs are related to the transforming growth factor-β (TGF-β) family of extracellular signaling proteins. Members of the TGF-β/BMP family are signaling molecules that regulate a diverse range of cellular activities including differentiation, proliferation, apoptosis, migration, positional information, and stem cell renewal, although members of this large family of cytokines show specificities in signal transduction and cellular functions [25–29]. Many proteins within the BMP subgroup have specific abilities to induce chondrogenesis and osteogenesis, leading to endochondral bone formation [16]. Subsets of BMPs and their receptors play key roles in embryonic development and skeletal formation and are expressed in many adult tissues including skeletal muscles and chondrocytes. ACVR1 is expressed in chondrocytes and osteoblasts and a constitutively active form of ACVR1 (caALK2) enhances chondrogenesis and induces HO [30, 31].

BMP Pathway Activation by ACVR1/ALK2 R206H

Protein homology modeling of the mutant ACVR1/ALK2 proteins identified in classic and atypical FOP patients predicts that these altered receptors are likely to activate the ACVR1/ALK2 protein and enhance receptor signaling through both BMP-responsive and BMP-independent mechanisms [14•, 32–34]. These predictions are supported by in vitro assays that have shown that the ACVR1 R206H mutation is an activating mutation that stimulates BMP signaling without requiring BMP to initiate the signaling cascade [35, 36•, 37, 38].

In vitro data were supported by assays using zebrafish, a well-defined genetic model for studying BMP signaling activity. Zebrafish embryonic development, which is highly sensitive to variations in levels of BMP signaling, was monitored for response to mutant ACVR1 R206H receptors and showed the first confirmation of ligand-independent hyperactivation of BMP activation in vivo [36•]. Further analyses demonstrated that the mutant ACVR1 signaling is mediated through the BMP-Smad signaling pathway.

ACVR1 codon 206 is within the GS activation domain, a critical site within TGF-β/BMP type I receptors for binding and activation of the pathway-specific Smad signaling proteins. The GS domain is a specific binding site for FKBP1A (also known as FKBP12), a highly conserved inhibitory protein that prevents leaky activation of type I receptors in the absence of ligand [39]. Experimental data support that the ACVR1 R206H protein has reduced interaction with FKBP1A in the absence of BMP, and suggest that this impaired FKBP1A-ACVR1 interaction contributes to BMP-independent BMP pathway signaling [36•, 37, 38].

In humans, the mutation that causes FOP overactivates the BMP pathway, yet allows human embryonic development to occur relatively unimpaired, with only mild skeletal effects. One explanation may be that the FOP mutation is only moderately activating. Comparison of FOP ACVR1 R206H to constitutively active ACVR1 Q207D in micro-mass chondrogenesis assays demonstrated that the ACVR1 R206H receptor has a milder stimulation of cell differentiation compared to caACVR1 Q207D [36•]. These data are consistent with the idea

that in postnatal connective tissues, increased BMP signaling from the mutant receptor may be only moderately "on" under basal in vivo conditions allowing for the quiescent periods that are observed in patients between active episodes of heterotopic bone formation, but priming the cells to respond to changes in the local tissue environment, such as tissue injury, by forming extraskeletal bone.

Formation of Heterotopic Lesions in FOP

The usual progression of postnatal HO in FOP begins during early childhood with progression that follows predictable temporal and spatial patterns [10, 11]. In the absence of trauma, which alters the natural progression of the disease, episodes of heterotopic bone formation (or "flare-ups") appear to initiate spontaneously and show initial involvement of the upper back and neck, with subsequent involvement of the lower trunk and the limbs. Over time, ectopic bone formation in FOP is progressive, cumulative, and extensive, bridging the joints of the axial and appendicular skeleton and causing nearly complete immobilization of the body.

Episodes of HO in FOP are frequently induced by soft tissue injury, such as surgery, muscle fatigue, intramuscular injections, preschool immunizations, and viral illnesses [10, 11]. All of these stimuli have in common a robust inflammatory contribution by the highly conserved innate immune wound-response system. Histologic evaluation of rare patient biopsy samples [40, 41], as well as of spontaneous lesions formed in an FOP knock-in mouse model (Shore, Unpublished observations) support that inflammation and an immune response play a role in the early stages of the bone formation process in this disease. Inflammatory cells of lymphocytic, macrophage, and mast cell origin are present during an immune response that is similar to, but greater in magnitude than, a normal tissue response to injury [40, 42, 43] and accompanies the destruction of the surrounding connective tissues.

The initial phase of tissue turnover in FOP lesion formation precedes a phase of tissue formation. An angiogenic and robust fibroproliferative stage is followed by production of newly formed cartilage and bone through a normal sequence of endochondral ossification stages [40, 41, 44, 45]. In essence, one tissue is replaced by another.

The presence of inflammatory cells is associated with damage to skeletal muscle cells and initial stages of a tissue repair response include a hypoxic microenvironment. Protein structure homology modeling of the mutant R206H ACVR1/ALK2 receptor predicts that an aberrant salt bridge would promote overactivity of the FOP receptor under hypoxic conditions and lower pH [33] and a hypoxic tissue environment has been associated with induction of HO in a mouse model [46]. Our preliminary studies [47] tested the hypothesis that a hypoxic microenvironment enhances signaling through the mutant ACVR1/ALK2 receptor and demonstrated that signaling was enhanced and of longer duration in the presence of the ACVR1 R206H mutation under hypoxic conditions (1% O_2) compared with standard (21% O_2).

In recent years, there has been a growing interest in identifying the osteoprogenitor cells that participate in heterotopic bone formation. A number of sources of putative progenitor cells have been identified, but few studies have investigated the identity of the specific cell lineages responsible for the chondro-osseous anlagen BMP-associated HO.

Early FOP lesions are highly angiogenic [44] and recent investigations of FOP patients and in vivo mouse models of FOP-like HO demonstrated that connective tissue progenitor cells of endothelial origin contribute to multiple stages in the development of the heterotopic anlagen [48–50]. Although the data support that endothelial cells are not the only source of

chondro/osseous progenitor cells that contribute to HO, such cells appear to have a significant role in this process.

Given the multiple roles of the BMP signaling pathway in many stages of development and in a wide ranges of cells and tissues, it would not be unexpected that the enhanced BMP signaling induced by the ACVR1/ALK2 mutation in FOP contributes to multiple stages of hetero-topic bone formation, including an aberrant response to tissue injury that induces an environment that supports and promotes bone formation, recruitment of progenitor cells, and directing cell differentiation to cartilage and bone fates.

Conclusions

The identification of mutations in ACVR1/ALK2, a BMP receptor, has focused attention on the roles of the BMP signaling pathway in regulating normal and pathologic events leading to new bone formation. This discovery provides opportunities to discover the specific cellular mechanisms that regulate bone and cartilage cell differentiation and that direct the formation of the skeleton, with wide implications to improving human health and treating disease. Recent investigations are elucidating the effects of FOP-causing mutations on signaling through the mutated receptors, as well as elucidating the identities of adult tissue stem cells that can be recruited to form cartilage and bone, and ultimately determining the changes in cells and tissues that support the initiation and progression of bone-forming events. As our understanding of HO in patients with FOP continues to expand, we will also gain knowledge that can be applied to the more common forms of acquired HO as well as other disorders of bone formation.

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