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The FOP Metamorphogene Encodes a Novel Type I Receptor That Dysregulates BMP Signaling

Frederick S. Kaplan, M.D.,

Departments of Orthopaedic Surgery and Medicine, and The Center for Research in FOP & Related Disorders, The University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Robert J. Pignolo, M.D., Ph.D., and

Department of Medicine, and The Center for Research in FOP & Related Disorders, The University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Eileen M. Shore, Ph.D.

Departments of Orthopaedic Surgery and Genetics, and The Center for Research in FOP & Related Disorders, The University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Abstract

The ability of mature organisms to stabilize phenotypes has enormous selective advantage across all phyla, but the mechanisms have been largely unexplored. Individuals with fibrodysplasia ossificans progressiva (FOP), a rare genetic disorder of progressive heterotopic ossification, undergo a pathological metamorphosis in which one normal tissue is transformed into another through a highly regulated process of tissue destruction and phenotype reassignment. This disabling metamorphosis is mediated by the FOP metamorphogene, which encodes a mutant bone morphogenetic protein (BMP) type I receptor that exhibits mild constitutive activity during development and severe episodic dysregulation postnatally. The discovery of the FOP metamorphogene reveals a highly conserved target for drug development and identifies a fundamental defect in the BMP signaling pathway that when triggered by injury and inflammation transforms one tissue into another.

Keywords

Fibrodysplasia ossificans progressiva; heterotopic ossification; bone morphogenetic protein (BMP) receptor; BMP signaling; metamorphosis; metamorphogene; ACVR1; activin-like kinase 2 (ALK2)

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Corresponding author: Frederick S. Kaplan, M.D., Isaac & Rose Nassau Professor of Orthopaedic Molecular Medicine, Department of Orthopaedic Surgery & Medicine, and The Center for Research in FOP & Related Disorders, The University of Pennsylvania School of Medicine, C/o Hospital of The University of Pennsylvania, Silverstein Pavilion - Second Floor, 3400 Spruce Street, Philadelphia, PA 19104, USA, Tel: 215-349-8726, Fax: 215-349-5928, frederick.kaplan@uphs.upenn.edu.

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1. Introduction

1.1. FOP

FOP is a complex and disabling genetic disease characterized by congenital skeletal malformations and inflammation-induced extraskeletal bone formation through an endochondral process [1–3]. Heterotopic ossification begins in childhood, may occur without warning, or can be induced by trauma or various viral illnesses. Bone formation is episodic and progressive, leading to the formation of a heterotopic skeleton in well-defined spatial and temporal patterns [4–5]. Eventually, progressive ossification causes extraarticular ankylosis of nearly all axial and appendicular joints and permanent immobilization in a disabling “second skeleton” of heterotopic bone [1,2,4,6,7,8]. Misdiagnosis is common [9,10]. Death results most commonly by the fifth decade from complications of restrictive chest wall disease [11,12].

1.2. The BMP Signaling Pathway

A comprehensive understanding of the bone morphogenetic protein (BMP) signaling pathway is critical to understanding the pathophysiology of FOP. The bone morphogenetic proteins (BMPs) are a family of highly conserved extracellular signaling molecules that regulate progenitor cell fates [13–17].

BMPs act through complex biochemical signaling pathways and function in a wide variety of cells and tissues during embryonic development and postnatal life. BMPs signal by binding to and activating transmembrane heterotetrameric complexes of type I and type II BMP receptors. BMP signaling is mediated through three known type I receptors: BMPRIA/ALK3, BMPRIB/ALK6, and ACVR1/ALK2, and two type II receptors, BMPRII and ActRII. Until recently, BMPRIA/ALK3 and BMPRIB/ALK6 have been the overwhelming focus of most BMP type I receptor studies [13–16, 18–20].

A unique feature of all transforming growth factor- β /bone morphogenetic protein (TGF- β /BMP) type I receptors is a cytoplasmic juxtamembrane region rich in glycine and serine residues (GS domain). Following ligand binding, serines and threonines in this region are phosphorylated by the constitutively active BMP type II receptor, thus activating the BMP type I receptor to transmit signals through SMAD and mitogen-activated protein kinase (MAPK) signaling pathways to regulate nuclear transcription of BMP responsive target genes [13–19, 21–24].

The GS domain of all TGF- β /BMP type I receptors is a critical site for the activation of pathway-specific SMAD signaling proteins by constitutively active TGF- β /BMP type II receptors. Importantly, FKBP1A (also known as FKBP12), binds and stabilizes the inactive conformation of all TGF- β /BMP type I receptors including ACVR1/ALK2. When bound to the GS domain, FKBP1A prevents promiscuous or leaky activation of all type I receptors in the absence of ligand [25–28]. Importantly, FKBP1A also serves as a docking protein for SMAD/SMURF complexes that mediate ubiquitination, internalization, and degradation of all BMP type I receptors [29,30]. As a result, FKBP1A is predicted to regulate the steady-state concentration of all BMP type I receptors (ALKs 2, 3, 6) at the cell membrane.

1.3. Dysregulation of BMP Signaling in FOP Cells

Studies of the *Drosophila decapentaplegic* mutant, a dipteran model of dysregulated BMP signaling [31], as well as studies of the classic FOP phenotype, supported that the primary molecular pathology in FOP involved both embryonic patterning and postnatal response to injury. The association of these two developmentally associated processes strongly suggested that the bone morphogenetic protein (BMP) signaling pathway might be involved

in the pathogenesis of FOP [31]. A series of discoveries in *in vitro* and *in vivo* systems provided compelling evidence of profound dysregulation of the BMP signaling pathway in FOP cells.

These findings included but were not limited to:

- Increased expression of BMP4 [32–35]
- Failure to upregulate multiple BMP antagonists [36]
- Failure to appropriately regulate the concentration of BMP in the extracellular space [37,38]
- BMP expression in fibroproliferative cells of early FOP lesions [32]
- Incomplete modulation of BMP signaling by cell-surface heparan sulfate proteoglycans [39,40]
- Increased concentration of BMP type I receptors at the cell surface [38]
- Failure to appropriately internalize and degrade BMP type I receptors [37,38,41]
- Sustained BMP signaling in FOP cells in the absence and presence of BMP [38]
- Dysregulated BMP-independent signaling through the SMAD pathway [42,43]
- Dysregulated BMP-dependent signaling through both the SMAD and the p38 MAPK pathways [42,43]
- Enhanced osteogenic differentiation of FOP connective tissue progenitor cells [42]

2. The FOP Metamorphogene

2.1 The Discovery of the FOP Metamorphogene

The FOP gene was mapped by genome-wide linkage analysis to chromosome 2q23-24, a region that includes the gene encoding Activin receptor A type I/activin-like kinase 2 (ACVR1/ALK2), a type I serine-threonine kinase receptor initially reported to mediate activin signaling but shown more recently to be a BMP type I receptor [44]. ACVR1/ALK2 is one of seven activin-like kinases (ALKs) in humans and one of the three classic BMP type I receptors encoded in the human genome [45]. Within the linkage interval, ACVR1/ALK2 was a prime candidate gene for FOP based on a number of studies supporting dysregulated BMP signaling in the pathogenesis of FOP.

A heterozygous missense mutation (c.617G >A; R206H) was identified in the glycine-serine (GS) activation domain of ACVR1/ALK2 in all classically affected FOP patients worldwide [44]. This single nucleotide mutation transforms a morphogen receptor gene into a metamorphogene and provides a permissive genetic background for the developmental and post-natal features of classic FOP (Figure 1). The mutant ACVR1/ALK2 protein activates BMP signaling in the absence of BMP and permits robust signaling in the presence of BMP [46]. Identification of the mutant transmembrane receptor (remarkably containing a single substituted amino acid residue) provides the basis for elucidating the molecular pathophysiology of dysregulated BMP signaling and resultant skeletal metamorphosis in FOP [47].

Investigations of ACVR1/ALK2 function in embryonic development and in cell differentiation have been limited [48,49]. ACVR1/ALK2 is expressed in many tissues including skeletal muscle, blood vessels, and chondrocytes, providing support for its phenotypic effects in FOP including developmental joint malformations and postnatal heterotopic ossification consistent with the classic FOP phenotype [49]. Consistent with the

classic FOP phenotype, constitutive activation of ACVR1/ALK2 induces alkaline phosphatase activity in C2C12 muscle satellite cells, upregulates BMP4, downregulates BMP antagonists, fails to appropriately regulate the concentration of BMP in the extra cellular space, dysregulates BMP signaling through the SMAD pathway, expands cartilage elements, induces ectopic chondrogenesis and stimulates joint fusions, findings similar to those seen in FOP [44,46,49–51].

Constitutively active ACVR1/ALK2 expression in embryonic chick limb buds induces expansion of chondrogenic anlage and leads to joint fusions, suggesting that promiscuous ACVR1/ALK2 signaling alters cell fate and induces undifferentiated mesenchyme to form cartilage and bone [46,49]. In the chick, however, heterotopic bone is seen at birth, whereas in humans with FOP, heterotopic bone is not seen at birth, and occurs later in childhood after an inflammatory trigger. These findings suggest that the mutant ACVR1/ALK2 receptor in FOP is only mildly constitutively active and responsive to receptor stimulation. Recent data support these findings [42,46]. Enhanced ACVR1/ALK2 activation in FOP is also supported by recent data showing increased BMP pathway-specific SMAD phosphorylation and expression of BMP transcriptional targets in FOP cells, as well as rescue of an ACVR1/ALK2 loss of function phenotype by mutant FOP ACVR1/ALK2 in zebrafish embryos [46].

Recent studies examined BMP signaling and osteogenic differentiation using connective tissue progenitor cells (SHED cells; stem cells from human exfoliated deciduous teeth) from discarded primary teeth of FOP patients and controls [42].

SHED cells from unaffected individuals transmitted BMP signals through both the SMAD and p38 mitogen-activated protein kinase (MAPK) limbs of the BMP signaling pathways and responded to BMP4 treatment by inducing BMP responsive genes. FOP SHED cells showed BMP-independent signaling as well as enhanced responsiveness to BMP stimulation. Further, FOP SHED cells showed more rapid differentiation to an osteogenic phenotype than control cells [42]. These data strongly support that both BMP-independent and BMP-dependent signaling occur as a result of mutant ACVR1/ALK2 activity in FOP cells. These data substantially extend our understanding of dysregulated BMP signaling in a progenitor cell population relevant to the pathogenesis of FOP [42].

2.2 A Novel Protein Encoded by the FOP Metamorphogene

Many abnormalities in the BMP signaling pathway were unveiled before the discovery of the FOP gene, and suggested that (BMPRIA/ALK3) may also be involved in the FOP disease process [38]. While still unresolved, these data are compatible with recent animal models of BMP signaling in which FOP features have been observed. In two classic model systems (Drosophila and Zebrafish), ACVR1/ALK2 homologues (Saxophone and Alk8), do not act independently, but rather in heteromeric complexes with other BMP type I receptors (Thickveins and Alk8), which are the Drosophila and Zebrafish orthologs of BMPRIA/ALK3 and human ACVR1/ALK2 respectively [52,53]. These data strongly suggest that the abnormal cellular metabolism of BMPRIA/ALK3 in FOP may arise from its association with ACVR1/ALK2 in heteromeric signaling complexes [52,53].

Protein homology modeling of the mutant FOP receptor predicts changes in both BMP-independent and BMP-dependent signaling in FOP cells [44,54]. Protein modeling of the FOP mutation predicts impaired FKBP1A (FKBP12) binding and/or activity with resultant BMP-independent as well as enhanced BMP-dependent signaling, coordinate features of BMP signal dysregulation that have recently been demonstrated in FOP cells [46,54]. Thus, it seems likely that the interaction of FKBP1A with the GS domain may be altered in FOP, leading in part to BMP-independent activity of ACVR1/ALK2. Recent preliminary data

strongly support this hypothesis [46]. Further, loss of autoinhibition of ACVR1/ALK2 is predicted to change not only the intensity, but also the duration of BMP signaling (42,55), thus creating new repertoires of BMP activity not previously observed in nature.

2.3. Inflammation & the FOP Metamorphogene

The recurrent heterozygous ACVR1/ALK2 (R206H) mutation that causes FOP creates a novel form of the receptor that increases the risk of FOP to 100% for all affected individuals (44). Interestingly, while this mutation is necessary for FOP and may be directly responsible for the congenital malformations in the condition, it does not appear sufficient for the disabling, episodic, postnatal flare-ups of heterotopic ossification (56–59). Activation of FOP by the innate immune system appears to be an important trigger for post-natal flare-ups of the disease (60–64).

Presently five animal models reproduce various clinical and pathological features of FOP-like heterotopic ossification [61–67]. One in particular, an ACVR1/ALK2 mutant knock-in chimera, reproduces all of the phenotypic features of FOP and provides proof-of-concept that the canonical FOP metamorphogene causes all of the phenotypic features of classic FOP and with patterns and triggers similar to that in the human condition [65].

Four recent *in vivo* studies have examined the role of inflammation in triggering heterotopic ossification in a BMP-permissive environment [61–64]. In a unique FOP case study and associated murine bone marrow transplantation experiment, the innate immune system was required for disease flare-ups [62]. In a transgenic mouse model of BMP-induced heterotopic ossification, the activity of circulating monocytes and tissue macrophages was inhibited pharmacologically and genetically, and found to substantially abrogate inflammation-associated heterotopic ossification [61]. In a related animal study, local inflammation was sufficient to stimulate heterotopic ossification in a transgenic mouse model in which BMP4 was overexpressed at the neuromuscular junction [63]. In yet another study, the conditional activation of inflammatory pathways in a constitutively active ALK2 mouse model led to the activation of heterotopic ossification at sites of inflammation [64]. Together, these findings support that dysregulation of the BMP signaling pathway and an inflammatory microenvironment are both required for the formation of heterotopic ossification.

2.4. Developmental Effects of the FOP Metamorphogene

In addition to its effect on connective tissue metamorphosis, the FOP metamorphogene leads to a wide range of phenotype abnormalities during development and postnatal life including:

- Dysregulated skeletal morphogenesis [1,68]
- Benign skeletal neoplasms [69]
- Degenerative joint disease [1,68]

The congenital phenotypes caused by the FOP metamorphogene include an array of congenital malformations and skeletal anomalies including malformed great toes, fusion of the cervical facet joints, fusion of the costovertebral joints, proximal medial tibial osteochondromas, and short broad femoral necks [1,68–72]. Unlike post-natal heterotopic ossification, these anomalies all seem to occur without a histologically observable inflammatory component, suggesting that an inflammatory trigger may not be necessary for the congenital features of the disease.

Interestingly, identical twins with FOP have nearly identical congenital malformations, but have a divergent history of postnatal heterotopic ossification that more closely follows

variable and individual postnatal inflammatory triggers such as trauma and viral illnesses [60]. Importantly, BMP signaling stimulates chondrocyte differentiation at multiple steps during embryonic cartilage development [73]. Profound over-activity of the BMP signaling pathway, as seen in Noggin Knockout mice in the absence of inflammatory triggers, leads to failure of formation of all diarthrodial joints [74]. Taken together, it is reasonable to hypothesize that the formation and maintenance of diarthrodial joints is sensitive to various gradients of BMP activity whereas postnatal heterotopic ossification is more dependent on inflammatory stimuli in the setting of dysregulated BMP signaling. A more complete analysis of the developmental effects of the FOP metamorphogene may be found in several recent articles and reviews [68–71].

2.5. Hypoxia & the FOP Metamorphogene

Hypoxia is predicted to increase BMP signaling from the mutant ACVR1/ALK2 receptor in both a pH-independent and a pH-dependent manner. Preliminary data support this hypothesis [75]. Importantly, generation of a hypoxic microenvironment triggered by BMP2 in skeletal muscle has recently been shown to be a critical step in the formation of heterotopic bone in a murine model [76]. To better understand the molecular constraints and physiological implications of an hypoxic microenvironment on the FOP mutation, detailed *in silico* modeling of wild type and mutant ACVR1/ALK2 were undertaken [54].

In silico modeling predicts that in the wild-type ACVR1/ALK2 model, the conserved arginine at codon 206 forms a salt bridge with an invariant aspartate residue at codon 269 [54]. In contrast, histidine at residue 206 (as occurs in FOP), would participate in a salt bridge with aspartate at residue 269 only at decreased intracellular pH and with extensive structural rearrangement predicting that substitution with histidine creates a pH-sensitive switch within the activation domain of the receptor [54]. For example, flare-ups of FOP might result from physiological changes such as trauma-associated hypoxia that lower intracellular pH and activate the switch. If so, perhaps, the intracellular pH of affected connective tissues could be modulated pharmacologically to suppress flare-ups of FOP or diminish the extent of heterotopic ossification [54].

Development of therapeutic approaches that abrogate hypoxic stress may serve to inhibit heterotopic bone formation in general, whether initiated by BMP ligand or by the aberrant ACVR1/ALK2 (R206H) receptor hypothesized to become hyperactive under conditions of hypoxia and/or intracellular acidosis [54]. Such approaches may prove useful for a wide array of non-genetic forms of heterotopic ossification.

2.6. FOP Variants & Novel Mutations in the FOP Metamorphogene

All patients with classic clinical features of FOP (great toe malformations and progressive heterotopic ossification) carry the same heterozygous mutation (c.617G>A; R206H) in the glycine-serine residue (GS) activation domain of activin A type I receptor/activin-like kinase 2 (ACVR1/ALK2). However, among patients with FOP-like heterotopic ossification and/or toe malformations, we also identified patients with clinical features unusual for FOP. These atypical FOP patients form two classes: FOP-plus (classic defining features of FOP plus one or more atypical features) and FOP variants (major variations in one or both of the two classic defining features of FOP [68]. All patients in both classes have heterozygous ACVR1/ALK2 missense mutations in conserved amino acids in ACVR1/ALK2. While the canonical c.617G>A; R206H mutation was found in all cases of classic FOP and most cases of FOP-plus, novel ACVR1/ALK2 mutations occur in all FOP variants and in two cases of FOP-plus [68]. Protein structure homology modeling predicts that each of the amino acid substitutions seen in classic FOP or its variants activates the ACVR1/ALK2 protein to enhance receptor signaling [68]. We observed genotype-phenotype correlation between

some ACVR1/ALK2 mutations and the age of onset of heterotopic ossification or on embryonic skeletal development.

3. Skeletal Metamorphosis in FOP

3.1 FOP & Skeletal Metamorphosis

The ability of mature organisms to stabilize tissue phenotypes has enormous selective advantage. However, the mechanisms by which tissues and organs retain their distinct phenotypic stability throughout adult life have been shrouded in mystery. FOP exemplifies a pathological process of metamorphosis, the promiscuous transformation of one normal tissue or organ into another - not through the transdifferentiation of one mature cell into another [77], but rather through a pathological process in which the normal structure and function of one tissue or organ is destroyed and replaced with that of another tissue or organ [47].

The histologic stages of skeletal metamorphosis in FOP have been well-described [47,66,78–80]. Skeletal metamorphosis in FOP begins with a catabolic phase in which a soft tissue injury triggers an inflammatory mononuclear cell infiltrate, and leads to widespread muscle cell injury and death. This catabolic phase is followed rapidly by an anabolic phase in which a highly angiogenic fibroproliferative lesion forms and matures through an endochondral process that culminates in the formation of a new heterotopic bone. As the process of metamorphosis continues and spreads to contiguous and adjacent sites, the new skeletal elements ramify to form a disabling second skeleton of heterotopic bone.

Inflammatory signals appear to be required for the initiation of skeletal regeneration and heterotopic ossification [61–64]. Cells of the innate immune system derived from hematopoietic precursors have been implicated in the skeletal metamorphosis of FOP [62]. However, the definitive contribution of hematopoietic cells to the pathogenesis of skeletal metamorphosis has, until recently, remained obscure. Examination of a patient with FOP who coincidentally developed intercurrent aplastic anemia demonstrated that bone marrow transplantation does not cure FOP, most likely because the hematopoietic cell population is not the target, or at least not the dominant target, of the intrinsic dysregulation of the BMP signaling pathway in FOP. However, following transplantation of bone marrow from a normal donor, immunosuppression of the immune system appeared to ameliorate activation of skeletal metamorphosis in the genetically susceptible chimeric host. Moreover, even a normal functioning innate immune system is apparently sufficient to trigger an FOP flare-up in the genetically susceptible chimera [62]. Thus, innate immune cells of hematopoietic origin may be required to trigger FOP lesions, but even normal cells of the innate immune system may be capable of doing this in an individual who harbors connective tissue progenitor cells that express the FOP metamorphogene [62].

Which connective tissue cells contribute to the fibroproliferative and chondrogenic mesenchymal anlagen in skeletal metamorphosis? Recent studies performed by two independent routes of investigation support that such cells arise from connective tissue progenitor cells that are, at least in part, of vascular origin [63,81]. The development of a knock-in mouse that replicates the identical mutation of classic FOP will facilitate the identification of the connective tissue progenitor cell(s) that is sensitive to dysregulated BMP signaling in FOP [65]. Such work is underway.

3.2. Principles of Skeletal Metamorphosis in FOP

A number of recent clinical and experimental observations, bone marrow transplantation studies, and progenitor lineage tracing experiments have elucidated a set of principles that guide our understanding of the process of metamorphosis in FOP and in animal models of

the condition [61–64,82,83]. While basal activity of the mutant ACVR1/ALK2 receptor may be sufficient to drive the nearly invariant array of skeletal malformations seen in FOP patients, it appears insufficient to trigger episodic flare-ups of FOP that lead to progressive heterotopic ossification and cumulative disability. Data from FOP patients and from *in vivo* animal models of FOP strongly suggest that inflammatory signals, in response to soft tissue injury, mobilize resident connective tissue progenitor cells of vascular origin that contribute to every stage in the development of the heterotopic anlagen. These principles are summarized below and are depicted in Figure 2:

1. Inflammatory cells of hematopoietic origin trigger soft tissue metamorphosis to heterotopic bone [61,62].
2. Inflammatory signals, in response to soft tissue injury, and in part from inflammatory cells, are sufficient to induce the process of heterotopic ossification in a BMP-conductive environment [61–64].
3. Cells of the innate immune system, specifically of the monocyte/macrophage lineage, induce skeletal metamorphosis in a BMP conductive environment [61].
4. Cells of the adaptive immune system, specifically of the lymphocyte lineage, propagate the growth and expansion of metamorphic changes in a BMP conductive environment [61].
5. Immunosuppression ameliorates heterotopic ossification in a genetically susceptible host [62,64].
6. Bone marrow transplantation does not cure FOP and is ineffective in abrogating its progression, because even normal inflammatory cells are sufficient to trigger FOP in a genetically susceptible host [62].
7. Circulating osteoprogenitor cells of hematopoietic origin may seed areas of evolving endochondral ossification [82].
8. Inflammatory signals, in response to soft tissue injury, mobilize resident connective tissue progenitor cells of vascular origin that contribute to every stage in the development of the heterotopic anlagen [62,63,83].
9. Connective tissue progenitor cells of vascular origin transduce inflammatory signals in a BMP-conductive environment and contribute to every stage in the evolution of heterotopic anlagen [63].
10. Therapeutic regulation of progenitor cell population involved in FOP lesions holds promise for treatment of FOP and possibly other disorders of heterotopic ossification [63].

3.3. Prevention & Treatment of Skeletal Metamorphosis in FOP

The discovery of the FOP metamorphogene along with emerging insights into the pathophysiology of ACVR1/ALK2-mediated heterotopic ossification reveal at least four plausible approaches to the treatment and/or prevention of FOP, and possibly even more common forms of heterotopic ossification [2,45,84]. These approaches include:

1. Blocking the activity of the mutant FOP receptor
2. Blocking the inflammatory triggers
3. Blocking the responding connective tissue progenitor (CTP) cells
4. Altering the inductive and/or conductive microenvironments that promote heterotopic ossification.

Several recent articles provide a thorough review of this important topic [2,45,71,85]. Here, we will briefly highlight only one limited approach, that of blocking the mutant receptor using signal transduction inhibitors or siRNA.

3.3.1. Signal Transduction Inhibitor (STI) Strategy—Small molecule signal transduction inhibitors (STIs) are important molecular tools for investigating signal transduction pathways, and have the potential for development into powerful therapeutic agents [85–87]. Residues close to the ATP-binding site of ACVR1/ALK2 are being exploited to achieve selectivity, even among closely related receptor serine-threonine kinases such as BMPRIA/ALK3, BMPRIB/ALK6 and ACVR1/ALK2 [88]. Small soluble molecular inhibitors designed to specifically block ACVR1/ALK2 must have sufficient specificity, efficacy, tolerance to resistance, as well as acceptable safety profiles before they can be used in clinical trials of FOP.

Recently, Dorsomorphin, a small molecule STI was identified in a screen for compounds that perturb dorsoventral axis formation in zebrafish [89]. Dorsomorphin and its derivatives inhibit all type I BMP receptors including ACVR1/ALK2, BMPRIA/ALK3, and BMPRIA/ALK6, and thus block BMP-mediated SMAD phosphorylation, target gene transcription, and osteogenic differentiation as shown in both *in vitro* and *in vivo* studies [46,51,64]. Additionally, Dorsomorphin blocks all BMP-specific SMAD signaling in FOP cells [46,51,72]. However, a safe and effective STI for FOP will likely inhibit ACVR1/ALK2 preferentially over ALK3 and ALK6. Also, while Dorsomorphin and its derivatives are able to inhibit SMAD activation, they have no appreciable effect on the ability of BMPs to activate p38 MAP kinases [89,90], a limb of the BMP signaling pathway that may plausibly play a role in the pathophysiology of FOP [43].

Compounds such as Dorsomorphin and its derivatives, as well as other more recently screened STIs, offer promise as pharmaceutical agents in the treatment of FOP and related disorders of heterotopic ossification [85]. Additional studies on BMP signaling will need to be evaluated in *in vivo* models of classic FOP to determine potential efficacy of this class of molecules in preventing inflammation-induced flare-ups of the condition.

3.3.2. Small Inhibitory RNA (siRNA) Strategy—Classic FOP results from a missense activating mutation in one of the two alleles of ACVR1/ALK2. Thus, strategies to specifically inhibit messenger RNA from the mutant allele while leaving messenger RNA from the normal allele unperturbed are desirable. Studies are underway to investigate the potential utility of a small inhibitory RNA (siRNA) strategy for treating FOP.

4. Concluding Remarks

FOP is a rare and illustrative genetic disorder that provides unique insight into the molecular, genetic and cellular principles of phenotype stability and metamorphosis. The single nucleotide heterozygous missense mutation that causes classic FOP in all affected individuals creates a metamorphogene that profoundly dysregulates BMP signaling in a highly conserved pathway involved in tissue development and repair. FOP is particularly instructive because it is caused by activating mutations in a BMP type I receptor that vastly alters not only body plan, but also tissue response to injury [91]. It is rare and perhaps unique that a single nucleotide mutation so vastly alters the identity of tissues and organ systems as a result of an inflammatory trigger and permits their promiscuous metamorphosis into other tissues and organs.

Identification of the molecular pathophysiology of the FOP metamorphogene reveals a critical and novel regulator of chondro-osseous differentiation that affects many

physiological processes including morphogenesis, metamorphosis, and tissue maintenance and repair. The FOP metamorphogene and its encoded receptor are not only therapeutic targets for FOP, but also an important focal point for understanding a wide variety of developmental and repair processes under the control of BMP signaling, and for understanding the fundamental processes that regulate tissue stability in vertebrate tissue and organs.

As Thomas Maeder wrote, “FOP and its problems lie at the crossroads of several seemingly unrelated disciplines. Answers to questions that FOP poses will also address grander issues of how the body first creates its shape and then knows where to stop, how tissues decide to become what they are, and why they don’t turn into something else (Figure 2) [92].

As William Harvey, the discoverer of the circulatory system, noted, “Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature, by the careful investigation of cases of rarer forms of disease. For it has been found in all things, that what they contain of useful or of applicable (nature) is hardly perceived unless we are deprived of them or they become deranged in some way [87].”

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Biographies

Frederick S. Kaplan, M.D. is The Isaac & Rose Nassau Professor of Orthopaedic Molecular Medicine, Chief of the Division of Orthopaedic Molecular Medicine, and Director of The Center for Research in FOP & Related Disorders at the University of Pennsylvania. He is an alumnus of The Johns Hopkins University (1972), and The Johns Hopkins University School of Medicine (1976), and was Chief Resident in Orthopaedic Surgery at The Hospital of The University of Pennsylvania and The Children's Hospital of Philadelphia from 1980 to 1981.

Kaplan was a Hartford Foundation Research Fellow in human genetics and molecular biology from 1989 to 1991 in the laboratory of his mentor and friend Dr. Michael Zasloff at The Children's Hospital of Philadelphia. This experience led to his exploration of the mechanisms for heterotopic bone formation and skeletal metamorphosis in several disabling childhood diseases and to identifying the molecular pathology of fibrodysplasia ossificans progressiva (FOP). Kaplan also identified another genetic bone disorder of children, progressive osseous heteroplasia (POH) and co-discovered the genes that cause both FOP and POH. His lifelong research goals are to develop more effective treatments and a cure for FOP and POH.

Kaplan is the chief medical and scientific advisor of The International FOP Association, The Progressive Osseous Heteroplasia Association, and The Melorheostosis Association. He serves on the Board of Directors of The Paget Foundation.

Kaplan has received numerous international awards and honors. In July 2006, he was named one of 15 Great Americans by NEWSWEEK.

Robert J. Pignolo, MD, PhD, is Assistant Professor in the Department of Medicine, Director of the Ralston-Penn Clinic for Osteoporosis and Related Bone Disorders, and the Ian Cali Clinical & Research Scholar in the Center for Research in FOP and Related Disorders at the University of Pennsylvania School of Medicine. Dr. Pignolo is a clinician-scientist with expertise in metabolic bone disease, gerontology, geriatric medicine, genetic and non-hereditary heterotopic ossification, as well as cell and molecular biology, especially related to cell proliferation, aging, and osteoblast differentiation. Dr. Pignolo's research focuses on osteoblast differentiation in conditions that reflect either impaired or inappropriate osteogenesis. He studies age-related bone loss and disorders of extraskeletal ossification, seemingly disparate conditions linked by the common thread of dysregulated osteoblast differentiation.

Eileen M. Shore, Ph.D. is an Associate Professor at the University of Pennsylvania School of Medicine in the Departments of Orthopaedic Surgery and Genetics, and the Co-Director of the Center for Research in FOP and Related Disorders. She is Scientific Advisor to the International Fibrodysplasia Ossificans Progressiva Association and the Progressive Osseous Heteroplasia Association. Dr. Shore is a long-standing and active member of the ASBMR and currently serves as President of the AIMM Board of Directors.

Dr. Shore attended the University of Notre Dame (B.S., Biology), Indiana University (M.A., Biology), and the University of Pennsylvania (Ph.D., Cell and Molecular Biology) with post-doctoral training at the Institute for Cancer Research, Fox Chase Cancer Center in Philadelphia.

Dr. Shore has applied her experience in molecular biology and genetics to her research interests in cell differentiation and development and their roles in human genetic disease. Her research focuses on fibrodysplasia ossificans progressiva (FOP) and progressive osseous heteroplasia (POH), two rare disorders characterized by *de novo* formation of bone. Dr. Shore's work led to the discovery of the mutated genes in both conditions. She currently investigates the cellular targets and molecular pathways involved in induction of bone formation with the goal of developing treatments for these and other more common bone disorders.

Figure 1. The FOP metamorphogene and its major phenotypes

The FOP metamorphogene encodes a type I BMP receptor (ACVR1/ALK2; R206H) that dysregulates BMP signaling and accounts for all of the congenital and postnatal features of FOP including congenital malformations of the great toes, heterotopic endochondral ossification, and ectopic skeletogenesis in characteristic anatomic patterns.

Figure 2. Working model of heterotopic ossification in FOP

Injury to skeletal muscle and connective tissues leads to lymphocyte and monocyte invasion, macrophage and mast cell activation, tissue hypoxia, and upregulation of inflammatory cytokines and osteogenic factors that recruit vascular progenitor cells to form the heterotopic anlagen. Hypoxia and pro-inflammatory cytokines contribute to the Tie2+ angiogenic response. Hypoxia-related changes may further sensitize vascular progenitor cells in which the FOP metamorphogene is expressed. The inflammatory response to muscle injury, the ambient hypoxia, the presence of the promiscuously active FOP metamorphogene in vascular progenitor cells, and the cross-talk between cells of the innate and adaptive immune system stimulate the induction and propagation of an ectopic skeletal element. CTPs, connective tissue progenitor cells; HSC, hematopoietic stem cells; T, T-cells; B, B-cells; MA, mast cells; FP, fibroproliferative cells; CB, chondroblasts; OB, osteoblasts; PGE₂, prostaglandin E₂; TNFα, tumor necrosis factor-alpha; blue lines, hematopoietic-derived pathways; brown lines, CTP-derived pathways; blunt-end lines signify inhibitory pathways; arrows signify stimulatory pathways. (Modified from Lounev et al. *J Bone Joint Surg Am* 91: 652-663, 2009)