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Survey

The FOP metamorphogene encodes a novel type I receptor that dysregulates BMP signaling

Frederick S. Kaplan [a,*], Robert J. Pignolo [b], Eileen M. Shore [c]

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

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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.

Contents

* Corresponding author at: C/o Hospital of The University of Pennsylvania Silverstein Pavilion – Second Floor, 3400 Spruce Street, Philadelphia, PA 19104, USA. Tel.: +1 215 349 8726; fax: +1 215 349 5928.
E-mail address: frederick.kaplan@uphs.upenn.edu (F.S. Kaplan).

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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 [1,2,4,6–8]. 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 signaling pathway might be involved in the pathogenesis of FOP [31]. A series of discoveries 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 the 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 postnatal features of classic FOP (Fig. 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