linked by a transcriptional cofactor complex including CBP.

With regard to signaling pathway following elevation of cAMP level, the canonical PKA/CREB/CRE signaling pathway would represent a major pathway, as significant suppression of enhancing effects on BMP signaling by PKA inhibitor and CREB inhibitor was seen.

Downstream of the PKA pathway, we analyzed the role of CREB using CREB RNAi and dominant-negative inhibitor of the CREB plasmid. CREB RNAi and A-CREB significantly decreased the RLA enhanced by dbcAMP. Interestingly, inhibition of BMP signaling was observed when endogenous CREB was knocked down using RNAi (see Fig. 3A). These results indicate that endogenous CREB/CRE signaling is important for Smad-mediated BMP signaling and CRE-mediated enhancement.

Coimmunoprecipitation experiments showed binding of p-Smad1/5/8 to CBP. This result is in accordance with recent reports indicating interaction of CBP/p300 with Smads in both TGF-β and BMP signaling and enhanced transcriptional activity of the Smads complex [15–20].

In the promoter sequence of the *Id1* gene, the CRE and BRE are located in close proximity and are close upstream to the TATA box. BMP and cAMP stimulation might be expected to lead to phosphorylation of Smad1/5/8 by BMPRI and phosphorylation of CREB by PKA, which bind to the BRE and CRE respectively and form the transcriptional cofactor complex regulating transcriptional activity of RNA polymerase II to express the osteoblastic phenotype. As CBP reportedly displays histone acetyl transferase activity and facilitates the transcription of genes [21], the enhancing action of the CRE on the BRE might promote the recruitment of CBP by p-CREB to the transcriptional cofactor complex and CBP might work as a coactivator in Smad-mediated BMP signaling. Further investigations are required to clarify this hypothesis.

Intermittent injection of PTH is known to stimulate bone formation in animals and humans and is currently utilized for the treatment of osteoporosis [22–24]. The mechanism of action for the anabolic effect of daily subcutaneous injection of PTH or its active fragment (teriparatide) on bone has not been elucidated, despite numerous studies. The present results suggest that elevation of intracellular cAMP levels in osteoblastic cells by PTH binding to the PTH1R membrane receptor (a G-protein-coupled receptor that activates the PKA pathway through Gαs stimulation of cAMP production) might enhance endogenous BMP-mediated osteoblastic differentiation by elevated cAMP level. This finding offers a possible explanation for the anabolic effects of the PTH on bone. To date, cAMP-PKA is considered as the main pathway in PTH signaling for the stimulation of osteoblasts, so modulation of BMP signaling by PTH through this pathway represents an important subject of study to further elucidate the mechanisms of action for PTH on bone.

In conclusion, we have demonstrated that the anabolic effects of cAMP on BMP-induced osteoblastic differentiation are mediated through a cAMP-PKA/CREB/CRE signaling pathway. These observations add to the understanding of the osteogenic actions of BMP and form a molecular basis for the development of bone-anabolic drugs that activate the cAMP signaling pathway in osteoblasts, such as PTH.

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References

1. Miyazono K (1999) Signal transduction by bone morphogenetic protein receptors: functional roles of Smad proteins. Bone (NY) 25:91–93
2. Horiuchi H, Saito N, Kinoshita T, Wakabayashi S, Tsutsumimoto T, Takaoka K (2001) Enhancement of bone morphogenetic protein-2-induced new bone formation in mice by the phosphodiesterase inhibitor pentoxifylline. Bone (NY) 28:290–294
3. Tsutsumimoto T, Wakabayashi S, Kinoshita T, Horiuchi H, Takaoka K (2002) A phosphodiesterase inhibitor, pentoxifylline, enhances the bone morphogenetic protein-4 (BMP-4)-dependent differentiation of osteoprogenitor cells. Bone (NY) 31:396–401
4. Horiuchi H, Saito N, Kinoshita T, Wakabayashi S, Tsutsumimoto T, Otsuru S, Takaoka K (2004) Enhancement of recombinant human bone morphogenetic protein-2 (rhBMP-2)-induced new bone formation by concurrent treatment with parathyroid hormone and a phosphodiesterase inhibitor, pentoxifylline. J Bone Miner Metab 22:329–334
5. Sasaoka R, Terai H, Toyoda H, Imai Y, Sugama R, Takaoka K (2004) A prostanoid receptor EP4 agonist enhances ectopic bone formation induced by recombinant human bone morphogenetic protein-2. Biochem Biophys Res Commun 318:704–709
6. Toyoda H, Terai H, Sasaoka R, Oda K, Takaoka K (2005) Augmentation of bone morphogenetic protein-induced bone mass by local delivery of a prostaglandin E EP4 receptor agonist. Bone (NY) 37:555–562
7. Sugama R, Koike T, Imai Y, Nomura-Furuwatari C, Takaoka K (2006) Bone morphogenetic protein activities are enhanced by 3′,5′-cyclic adenosine monophosphate through suppression of Smad6 expression in osteoprogenitor cells. Bone (NY) 38:206–214
8. Akiyama S, Katagiri T, Namiki M, Yamaji N, Yamamoto N, Miyama K, Shibuya H, Ueno N, Wozney JM, Suda T (1997) Constitutively active BMP type I receptors transduce BMP-2 signals without the ligand in C2C12 myoblasts. Exp Cell Res 235: 362–369
9. Katagiri T, Imada M, Yanai T, Suda T, Takahashi N, Kamijo R (2002) Identification of a BMP-responsive element in Id1, the gene for inhibition of myogenesis. Genes Cells 7:949–960
10. Lopez-Rovira T, Chalaux E, Massague J, Rosa JL, Ventura F (2002) Direct binding of Smad1 and Smad4 to two distinct motifs mediates bone morphogenetic protein-specific transcriptional activation of Id1 gene. J Biol Chem 277:3176–3185
11. Korchynskyi O, ten Dijke P (2002) Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. J Biol Chem 277:4883–4891
12. Takaoka K, Yoshikawa H, Hashimoto J, Ono K, Matsui M, Nakazato H (1994) Transfilter bone induction by Chinese hamster ovary (CHO) cells transfected by DNA encoding bone morphogenetic protein-4. Clin Orthop Relat Res 300:269–273
13. Ahn S, Olive M, Aggarwal S, Krylov D, Ginty DD, Vinson C (1998) A dominant-negative inhibitor of CREB reveals that it is a general mediator of stimulus-dependent transcription of c-fos. Mol Cell Biol 18:967–977
14. Nakagawa K, Imai Y, Ohta Y, Takaoka K (2007) Prostaglandin E_2 EP4 agonist (ONO-4819) accelerates BMP-induced osteoblastic differentiation. Bone (NY) 41:543–548

15. Nishihara A, Hanai JI, Okamoto N, Yanagisawa J, Kato S, Miyazono K, Kawabata M (1998) Role of p300, a transcriptional coactivator, in signalling of TGF-beta. Genes Cells 3:613–623
16. Feng XH, Zhang Y, Wu RY, Derynck R (1998) The tumor suppressor Smad4/DPC4 and transcriptional adaptor CBP/p300 are coactivators for smad3 in TGF-beta-induced transcriptional activation. Genes Dev 12:2153–2163
17. Janknecht R, Wells NJ, Hunter T (1998) TGF-beta-stimulated cooperation of smad proteins with the coactivators CBP/p300. Genes Dev 12:2114–2119
18. Pouponnot C, Jayaraman L, Massague J (1998) Physical and functional interaction of SMADs and p300/CBP. J Biol Chem 273:22865–22868
19. Furumatsu T, Tsuda M, Taniguchi N, Tajima Y, Asahara H (2005) Smad3 induces chondrogenesis through the activation of SOX9 via CREB-binding protein/p300 recruitment. J Biol Chem 280:8343–8350
20. Ghosh-Choudhury N, Singha PK, Woodruff K, Stclair P, Bsoul S, Werner SL, Ghosh-Choudhury G (2006) Concerted action of Smad and CREB-binding protein regulates bone morphogenetic protein-2-stimulated osteoblastic colony-stimulating factor-1 expression. J Biol Chem 281:20160–20170
21. Yuan LW, Gambee JE (2001) Histone acetylation by p300 is involved in CREB-mediated transcription on chromatin. Biochim Biophys Acta 1541:161–169
22. Dobnig H, Turner RT (1995) Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. Endocrinology 136:3632–3638
23. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH (2001) Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 344:1434–1441
24. Ishizuya T, Yokose S, Hori M, Noda T, Suda T, Yoshiki S, Yamaguchi A (1997) Parathyroid hormone exerts disparate effects on osteoblast differentiation depending on exposure time in rat osteoblastic cells. J Clin Invest 99:2961–2970

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REVIEW ARTICLE

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Skeletal metamorphosis in fibrodysplasia ossificans progressiva (FOP)

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Abstract Metamorphosis, the transformation of one normal tissue or organ system into another, is a biological process rarely studied in higher vertebrates or mammals, but exemplified pathologically by the extremely disabling autosomal dominant disorder fibrodysplasia ossificans progressiva (FOP). The recurrent single nucleotide missense mutation in the gene encoding activin receptor IA/activin-like kinase-2 (ACVR1/ALK2), a bone morphogenetic protein type I receptor that causes skeletal metamorphosis in all classically affected individuals worldwide, is the first identified human metamorphogene. Physiological studies of this metamorphogene are beginning to provide deep insight into a highly conserved signaling pathway that regulates tissue stability following morphogenesis, and that when damaged at a highly specific locus (c.617G > A; R206H), and triggered by an inflammatory stimulus permits the renegade metamorphosis of normal functioning connective tissue into a highly ramified skeleton of heterotopic bone. A comprehensive understanding of the process of skeletal metamorphosis, as revealed by the rare condition FOP, will lead to the development of more effective treatments for FOP and, possibly, for more common disorders of skeletal metamorphosis.

Key words morphogen · metamorphogene · ACVR1 · fibrodysplasia ossificans progressiva (FOP) · bone morphogenetic protein (BMP) · BMP receptor · heterotopic ossification

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Introduction

Cancer, aging, degeneration, inflammation, and repair are well-known tissue processes that have received enormous attention in the scientific and medical literature. In contrast, the process of metamorphosis, the transformation of one normal tissue or organ system into another through a pathological process, is virtually unexplored and has been hidden for centuries behind the mask of an exceedingly rare and catastrophic human disease, fibrodysplasia ossificans progressiva (FOP). The genetic and molecular lessons of FOP are rapidly being revealed and its importance to medicine far exceeds its rarity [1].

Clinical features of FOP

Two clinical features define classic FOP: congenital malformation of the great toes and progressive heterotopic ossification in distinct anatomic patterns (Fig. 1) [2]. Individuals with FOP appear normal at birth except for malformations of the great toes, which are present in 100% of classically affected individuals [3]. During the first decade of life, children with FOP develop painful and highly inflammatory

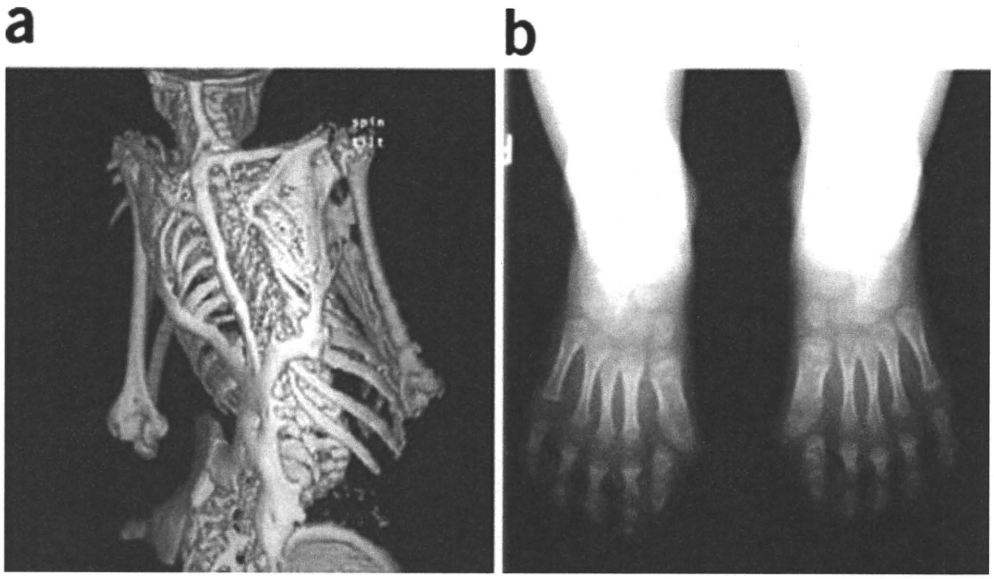

Fig. 1. Characteristic clinical features of fibrodysplasia ossificans progressiva (FOP). **a** Extensive heterotopic bone formation typical of FOP is seen by three-dimensional reconstructed computed tomography (CT scan) of the back of a 12-year-old child. **b** Anteroposterior radiograph of the feet of a 3-year-old child shows symmetrical great toe malformations. [Originally published in Shore EM, et al. (2006) Nat Genet 38:525–527. Copyright held by the authors]

soft tissue swellings (or flare-ups) that progressively and permanently transform soft connective tissues, including aponeuroses, fascia, ligaments, tendons, and skeletal muscles, into an armament-like encasement of heterotopic bone [4,5]. Ribbons, sheets, and plates of heterotopic bone replace skeletal muscles and connective tissues through a process of endochondral ossification that leads to the formation of a highly ramified second skeleton with resultant permanent immobility [6–9]. Minor trauma such as intramuscular immunizations, mandibular blocks for dental work, muscle fatigue, blunt muscle trauma from bumps, bruises, falls, or influenza-like illnesses can trigger painful new flare-ups of FOP, leading to progressive heterotopic ossification (HO) [10–14].

Surgical attempts to operatively remove heterotopic bone commonly lead to episodes of explosive and painful new bone growth [15–19]. HO in FOP progresses in characteristic anatomic and temporal patterns that mimic the patterns of normal embryonic skeletal formation. FOP involvement typically is seen first in the dorsal, axial, cranial, and proximal regions of the body and later in the ventral, appendicular, caudal, and distal regions [2,4,5,15,17]. Several skeletal muscles including the diaphragm, tongue, and extraocular muscles are enigmatically spared from FOP. Cardiac muscle and smooth muscle are not involved in the FOP process [2,15–17].

The clinical features of early lesional involvement in the axial regions are often different from those seen in the appendicular regions [20]. New lesions may appear rapidly. In the axial regions, swelling is often mistaken for tumors, as large bulbous lesional swellings may appear on the neck and back, whereas in the limbs, the swelling is often diffuse, and may be mistaken for acute thrombophlebitis, a complication that can occur in patients with FOP as a result of generalized immobility and associated venous stasis [20]. The qualitative differences in swelling in the axial versus the appendicular regions in patients with FOP may reflect regional differences in the anatomy of the subaponeurotic spaces as well as differences in the anatomy of the fascial compartments.

Bone formation in FOP is episodic, but disability is cumulative. Most patients with FOP are confined to a wheelchair by the third decade of life and require lifelong assistance in performing activities of daily living [2,15–19]. Severe weight loss may result from ankylosis of the jaw, and pneumonia or right-sided heart failure may complicate rigid fixation of the chest wall [21]. The severe disability of FOP results in low reproductive fitness, and fewer than ten multigenerational families are known worldwide [22]. The median age of survival is approximately 41 years, and death often results from complications of thoracic insufficiency syndrome [21].

Diagnosis and misdiagnosis of FOP

FOP is commonly misdiagnosed, as clinicians often fail to associate the rapidly developing soft tissue swellings that appear on the head, neck, and upper back with the malformed great toes [23]. FOP can be diagnosed clinically even before radiographic evidence of heterotopic ossification is seen if rapidly waxing and waning soft tissue lesions are associated with symmetrical malformations of the great toes [24]. When such associations are not made, FOP is commonly misdiagnosed as aggressive juvenile fibromatosis (extraabdominal desmoid tumors), lymphedema, or soft tissue sarcomas. Children often undergo unnecessary and harmful diagnostic biopsies that exacerbate the progression of the condition [23].

Additional skeletal anomalies in FOP

In addition to malformations of great toes and thumbs, early developmental anomalies are frequently observed in

the cervical spine [25]. Stiffness of the neck is an early finding in most patients and can precede the appearance of HO. Characteristic anomalies of the cervical spine include large posterior elements, tall narrow vertebral bodies, and fusion of the facet joints between C2 and C7, findings that are strikingly similar to those seen in mice with homozygous deletions of the gene encoding Noggin, a secreted bone morphogenetic protein (BMP) antagonist [26], supporting that increased BMP pathway signaling underlies the disease [25]. Although mutations in the Noggin gene have been reported [27–29], these findings could not be reconfirmed and are thought to be in error [30–34].

Other skeletal anomalies often associated with FOP include short malformed thumbs, clinodactyly, short broad femoral necks, and proximal medial tibial osteochondromas. The latter two findings are also seen in patients who have multiple hereditary exostoses (MHE), although the genes associated with multiple hereditary exostoses are not mutated in patients who have FOP. Nevertheless, these shared clinical findings may illuminate common pathway anomalies [2,15–17,35].

Epidemiological, genetic, and environmental factors in FOP

FOP is extremely rare, with a worldwide prevalence of approximately 1 in 2 million. There is no ethnic, racial, gender, or geographic predisposition [17,19]. Most cases arise as a result of a spontaneous new mutation. When observed, genetic transmission is autosomal dominant and can be inherited from either mothers or fathers [22,36].

Both genetic and environmental factors affect the phenotype of FOP. A study of three pairs of monozygotic twins with FOP found that, within each pair, congenital great toe malformations were identical. However, postnatal heterotopic ossification varied greatly depending on life history and environmental exposure. This study indicated that genetic determinants strongly influence disease phenotype during prenatal development and that environmental factors strongly influence postnatal progression of heterotopic ossification [37].

The pathobiology of skeletal metamorphosis in FOP

The process of skeletal metamorphosis, as exemplified by FOP, does not involve the transdifferentiation of one mature cell into another, but rather a pathological process in which the normal structure and function of one tissue or organ are destroyed and replaced by those of another functioning tissue or organ [9,38].

The pathological stages of skeletal metamorphosis have been well described in FOP and correspond to the BMP-induced lesions described in several reports [6–8,39–44]. Skeletal metamorphosis in FOP begins with a soft tissue injury that triggers an inflammatory infiltrate and proceeds to muscle cell injury and death, replacement with a highly angiogenic fibroproliferative lesion, and maturation through a cartilage anlagen that culminates in the formation of a new skeletal element [6–9,38–44].

Despite detailed descriptions of FOP pathology in the medical literature, there has been a persistent misconception in the medical and scientific community about the process. For example, it is commonly believed that the process of skeletal metamorphosis involves a transdifferentiation of mature muscles cells into bone cells. That, in fact, does not appear to occur in vivo. Studies by Katagiri and colleagues have documented that C2C12 myoblasts, which are believed to be derived from satellite cells in murine thigh muscle, can express an osteogenic phenotype in the presence of high concentrations of recombinant BMP or constitutively active BMP type I receptors in vitro, and provide the template for understanding how dysregulation of the BMP signaling pathway can lead to altered progenitor cell fates [45,46]. Thus, mature muscles cells including myofibers do not transdifferentiate into mature bone cells. Rather, normal muscle tissue is replaced by normal bone tissue in a complex pathological process of skeletal metamorphosis that involves the epigenetic reprogramming of progenitor cell fates.

Morphogen receptor genes

The bone morphogenetic protein (BMP) signaling pathway is one of the most highly conserved signaling pathways in nature and regulates a myriad of developmental and post-developmental processes beginning in early embryogenesis and continuing through adult life [41,47–54]. A large body of evidence supports that BMPs act as morphogens in vertebrate development [41,51–55]. BMPs signal by binding to and activating heterotetrameric transmembrane complexes of type I and type II BMP receptors. Both type I and type II BMP receptors are serine/threonine kinases that have similar functional domains. Ligand binding occurs preferentially at the N-terminal extracellular domain of the BMP receptors, which is connected by a single transmembrane region to the C-terminal cytoplasmic kinase domain [51–54]. BMP signaling can be mediated by four known type I receptors: TSR (ALK1), ACVR1 (ALK2), BMPR1A (ALK3), and BMPR1B (ALK6). A unique feature of type I receptors is a cytoplasmic juxtamembrane region rich in glycine and serine residues (GS domain). Following ligand binding, serines and threonines in the GS domain are phosphorylated by the constitutively active BMP type II receptor. The BMP type I receptor is activated by these phosphorylation events and transmits downstream BMP signals through BMP pathway-specific Smads (Smads1, –5, –8) and p38 MAPK signaling pathways to regulate transcription of BMP-responsive target genes. Numerous comprehensive reviews are available on this seminal signaling pathway that will orient the reader to the concepts which follow here [51–54].

Discovery of the FOP metamorphogene

A large body of work has supported dysregulated BMP signaling in the pathogenesis of FOP [56–63]. FOP was recently mapped to chromosome 2q23-24 by genome-wide linkage analysis, a region that includes the ACVR1 gene. ACVR1/ALK2 is one of seven activin-like kinases (ALKs) in the human genome that all encode transforming growth factor-beta (TGF-β)/BMP type I serine/threonine transmembrane receptors involved in the specification of cell fate and differentiation in a wide variety of cells and tissues during embryonic development and postnatal life [64]. In all classically affected individuals worldwide, an identical heterozygous missense activating mutation (c.617G > A; R206H) was subsequently identified in the glycine-serine (GS) activation domain of ACVR1 [3].

This single nucleotide missense mutation transforms a morphogen receptor gene into a metamorphogene. The resultant mutant protein alters the basal set point and ligand-dependent sensitivity for BMP signaling in a cell-autonomous manner in connective tissue progenitor cells [63]. Identification of the mutant transmembrane receptor (and remarkably a single substituted amino acid residue in that receptor) provides a basis for elucidating the molecular pathophysiology of dysregulated BMP signaling and resultant skeletal metamorphosis in this illustrative and disabling condition [3,63].

Although ACVR1/ALK2 has been recognized as a BMP receptor, investigations of its functions in embryonic development and in regulating cell differentiation have been limited [3]. ACVR1/ALK2 is expressed in many tissues including skeletal muscle and chondrocytes. Constitutive activation of ACVR1/ALK2 induces alkaline phosphatase activity in C2C12 cells, upregulates BMP4, downregulates BMP antagonists, expands cartilage elements, induces ectopic chondrogenesis, and stimulates joint fusions, findings nearly identical to those seen in FOP [65]. Constitutive ACVR1/ALK2 expression (similar to that seen in FOP) in embryonic chick limbs induces expansion of chondrogenic anlage and induces joint fusions, suggesting that promiscuous ACVR1/ALK2 signaling alters cell fate and induces undifferentiated mesenchyme to form cartilage and bone [65]. Enhanced ACVR1/ALK2 activation in FOP is supported by recent data showing increased pathway-specific Smad phosphorylation and expression of BMP transcriptional targets in FOP cells as well as reversal of an ACVR1/ALK2-homologue loss of function phenotype by the ACVR1 (FOP) construct in mutant zebrafish embryos [66,67].

Protein homology mapping of mutant ACVR1

Protein homology modeling of the mutant ACVR1/ALK2 in FOP predicts changes in both ligand-independent BMP signaling and in ligand-stimulated BMP signaling in FOP cells [68]. We hypothesize that the canonical FOP mutation (ACVR1 c.617G>A; R206H), which replaces an arginine residue with a histidine at amino acid 206 in the GS domain of ACVR1/ALK2, affects the binding and downstream function of coregulatory proteins.

The GS domain of all TGF-β BMP type I receptors is a critical site for activation of pathway-specific Smad signaling proteins by constitutively active TFG-β/BMP type II receptors [69–72]. FKBP12 binds and stabilizes the inactive confirmation of all type I TGF-β/BMP receptors including ACVR1/ALK2. When bound to the GS domain, FKBP12 prevents leaky activation of type I receptors in the absence of ligand [69–72]. Importantly, FKBP12 also serves as a docking protein for the Smad–Smurf complexes that mediate ubiquitination, internalization, and degradation of ACVR1/ALK2, and is predicted to regulate the concentration of ACVR1/ALK2 and its BMP type I receptor oligomerization partners at the cell membrane [73,74].

The FOP mutation is predicted to impair FKBP12 binding and/or activity with resultant increased basal activity of BMP signaling in the absence of ligand as well as hyper-responsiveness of BMP signaling following ligand binding, two features of BMP signal dysregulation that have recently been demonstrated in FOP cells [63]. Thus, one scenario is that FKBP12 interaction with the GS domain may be altered in FOP, leading to promiscuous ACVR1/ALK2 activity (Fig. 2). Recent preliminary data strongly support this hypothesis, and it is the subject of intensive investigation.

The immune system and metamorphosis in FOP

Although dysregulation of the BMP signaling pathway can explain many features of skeletal metamorphosis in FOP, other features of the disease strongly implicate an underlying inflammatory trigger and/or a conducive inflammatory microenvironment [75] (Fig. 3).

A recent study showed that aberrant expression of BMPs in soft tissue causes focal hypoxia and hypoxic stress within the target tissue, a prerequisite for the differentiation of stem cells to chondrocytes and subsequent formation of heterotopic bone [76]. Flare-ups of FOP are frequently associated with muscle fatigue and trauma-associated hypoxia [2,4,15,77]. Such physiological hypoxic stress is predicted to exacerbate tissue damage, to release inflammatory mediators and BMPs, and to mobilize free radicals and the subsequent stimulation of mutant ACVR1/ALK2 in as yet unidentified connective tissue progenitor cells that potentiate skeletal metamorphosis [76,78–84] (Fig. 3).

A major new area of FOP research is focused on the relationship of inflammatory triggers, mutant ACVR1/ALK2 receptors, and local environmental factors such as free radicals, pO_2, and pH in the episodic flare-ups of the disease [68,75,76,78–84]. Recent protein modeling studies by Groppe et al. predict that the canonical FOP mutation creates a pH-sensitive switch within the cytoplasmic domain of the mutant ACVR1/ALK2 receptor that leads not only to ligand-independent activation but also to ligand-dependent hyper-responsiveness of mutant ACVR1/

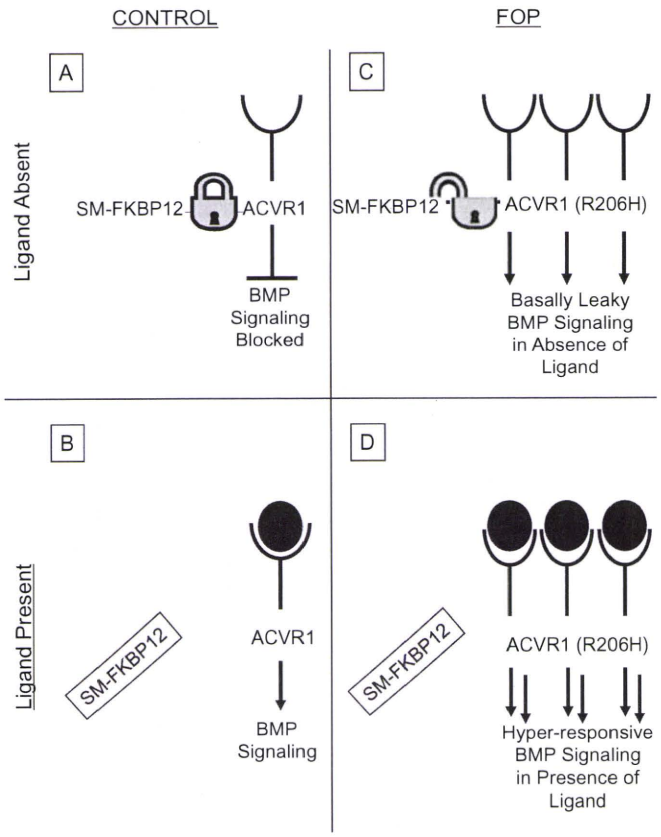

Fig. 2. Hypothetical schema of bone morphogenetic protein (BMP) signaling in FOP cells. [Adapted from Kaplan FS, et al (2008) Fibrodysplasia ossificans progressiva. Best Pract Res Clin Rheumatol, in press.] In control cells (**A**), in the absence of ligand, the Smad/Smurf-FKBP12 (SM-FKBP12) complex binds ACVR1 (a BMP type I receptor) and prevents its promiscuous phosphorylation by the constitutively active type II BMP receptor (not shown). SM-FKBP12 also promotes ubiquitin-associated degradation of ACVR1 in the absence of ligand, thus maintaining low steady-state levels of ACVR1 at the cell membrane. Following ligand binding in control cells (**B**), SM-FKBP12 inhibition is released, thus allowing the constitutively active BMP type II receptor (not shown) to phosphorylate ACVR1 and promote SMAD1, –5, –8 phosphorylation and downstream BMP signaling. In FOP cells, SM-FKBP12 does not bind appropriately to the mutant receptor [ACVR1 (R206)]. Thus, inhibition of BMP signaling is impaired in the absence of ligand, and basal leakiness of BMP signaling occurs (**C**). Additionally, because the SM-FKBP12 complex cannot properly target the mutant ACVR1 (R206H) receptor for ubiquitin-associated degradation, ACVR1 might be expected to accumulate at the cell surface. Thus, in the presence of ligand (**D**), hyper-responsive BMP signaling might be predicted to occur. *SM-FKBP12*, Smad/Smurf-FKBP12 complex; *arrows*, signaling promoted; (by FOP mutation); *open cups*, extracellular ligand-binding domain of ACVR1; *filled-in circles*, BMP; *filled-in circles inside of open cups*, BMP ligand binding to ACVR1

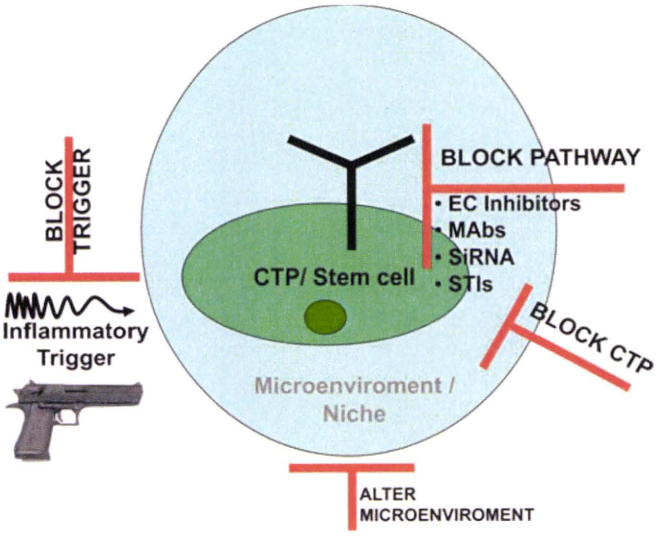

Fig. 3. Hypothetical approaches to prevention of skeletal metamorphosis in FOP. Four approaches to prevention of skeletal metamorphosis in FOP are diagrammed and include blocking the putative inflammatory trigger (*gun*), blocking the signal transduction pathway [using extracellular inhibitors, monoclonal antibodies (*MAbs*) against the renegade receptor, inhibitory RNA (*siRNA*) against the mutant receptor RNA, and signal transduction inhibitors (*STIs*)], blocking the repetitive connective tissue progenitor (*CTP*) cells that transduce the promiscuous signal, or blocking/altering the permissive microenvironmental niche. *EC*, extracellular

ALK2 in the microenvironment of a lowered intracellular pH [68].

Our working hypothesis on the pathophysiology of heterotopic skeletogenesis in FOP is that soft tissue injury creates an inflammatory and acidic tissue microenvironment in which prostaglandins, free radicals, and hypoxia in concert with inflammatory cells of hematopoietic origin stimulate resting connective tissue progenitor cells (CTPs) to become activated CTPs. These CTPs express the mutant ACVR1/ALK2 receptor that is putatively hyperactive in a mildly acidic intracellular environment and which results in misregulated BMP signaling through increased basal leakiness and conditional hyper-responsiveness to environmental BMPs [38] (Fig. 3). Once formed, the fibroproliferative cells in the FOP lesion produce robust amounts of BMP4, and overactivity of the BMP signaling is sufficient to drive the process of endochondral ossification to completeness in the absence of a continued inflammatory stimulus [38,58] (see Fig. 3).

The recent discovery of the FOP gene mutation in all familial and sporadic cases of classic FOP suggests a cell-autonomous basis for skeletal metamorphosis in FOP [61]. Protein modeling of the mutant receptor predicts destabilization of the GS activation domain consistent with basally leaky and conditional hyper-responsive BMP signaling as the cause of the ectopic chondrogenesis, osteogenesis, and joint fusion seen in FOP. Furthermore, these findings allow us to hypothesize that trauma and inflammation recruit osteogenic CTPs in which the activating mutation is expressed. These findings are consistent with in vivo observations in animal models showing that once the endochondral anlagen is induced, abrogation of the inflammatory response does not inhibit the formation of heterotopic bone [85].

Interactions between inflammatory cells and FOP mesenchymal cells in a microenvironment that is inductive and conducive to the initiation, progression, and sustenance of bone formation in FOP occurs at several steps (see Fig. 3). Recent studies have strongly suggested that migration of vascular progenitor cells to sites of inflammation is a key

step in normal and disordered regenerative responses and likely plays a role as well in the pathology of skeletal metamorphosis [85,86].

Stem cells and skeletal metamorphosis in FOP

Stem cells, progenitor cells, and inflammation must therefore lie at the very heart of the process of metamorphosis (Fig. 3). Hematopoietic cells have been implicated in the skeletal metamorphosis of FOP, and their replacement has been postulated as a possible cure [85]. However, the definitive contribution of hematopoietic cells to the pathogenesis of skeletal metamorphosis has, until recently, remained obscure. A recent study in a patient with FOP who coincidentally developed intercurrent aplastic anemia demonstrated that bone marrow transplantation does not cure FOP. However, following transplantation of bone marrow from a normal donor, immunosuppression of the immune system appeared to ameliorate activation of skeletal metamorphosis in a genetically susceptible host. Thus, cells of hematopoietic origin may contribute to and perhaps even trigger the formation of an ectopic skeleton, although they are not sufficient to complete the process alone. Moreover, even a normal functioning immune system is apparently sufficient to trigger an FOP flare-up in a genetically susceptible host [85].

Which cells contribute to the fibroproliferative and chondrogenic mesenchymal anlagen in skeletal metamorphosis? The question is fascinating, important, and unresolved. Recent studies performed with two independent routes of investigation support the contention that such cells are not of hematopoietic origin, but arise from a different pool of connective tissue progenitor (CTP) cells residing in skeletal muscle and associated connective tissues, with possible lineage origins in endothelial cells, smooth muscle cells, neural cells, or other CTP cells [85]. Therefore, multiple sources of pluripotent stem cells or progenitors may contribute to the formation of an ectopic skeleton in the process of skeletal metamorphosis [85]. Detailed lineage-tracing experiments in transgenic mice with stable lineage markers will be necessary to definitively determine the origin of these cells. The development of a knock-in mouse that replicates the identical mutation of classic FOP is presently underway and will facilitate the identification of the autonomous connective tissue progenitor cell(s) that contribute to dysregulated BMP signaling in FOP.

Taken together, skeletal metamorphosis appears to be a stem cell or progenitor cell disorder, triggered by inflammation. One normal tissue is replaced with another, but first, nearly all vestiges of the original tissue, except its soft tissue scaffolding, neurovascular infrastructure, and progenitor cell repository, are destroyed and then replaced with a different tissue and a different epigenetic identity. Recent studies in FOP suggest that connective tissue progenitor cells resident in the local tissues are responsible for this metamorphosis, although the process is triggered by local inflammatory signals [75,85]. Essentially, skeletal metamorphosis is a disorder of dysregulated tissue repair. Metamorphosis thus unmasks the deep developmental restraints that must exist in normal tissue repair, and which are liberated, to the detriment of the host, by the specific recurrent mutation in the ACVR1/ALK2 (R206H) metamorphogene [3,85].

Prevention and treatment of skeletal metamorphosis in FOP

Presently, medical intervention for FOP is supportive. Guidelines for symptomatic management of disease flare-ups have been published and highlight the anecdotal utility of glucocorticoids in managing new flare-ups affecting the function of major joints in the appendicular skeleton [87]. Nonsteroidal antiinflammatory medications, COX-2 inhibitors, leukotriene inhibitors, and mast cell stabilizers are useful anecdotally in managing chronic discomfort and ongoing flare-ups, but presently there is no proven efficacy with any therapy in altering the natural history of the disease [87]. The rarity, variable severity, and episodic clinical course of FOP pose substantial uncertainties when evaluating experimental therapies [87]. A recent report documented the failure of bone marrow transplantation to cure the condition, but suggested that chronic immunosuppression may have some utility, although its general use is not recommended [85].

Surgical release of joint contractures is generally unsuccessful and risks new, trauma-induced heterotopic ossification. Osteotomy of heterotopic bone or surgical removal of heterotopic bone to mobilize joints is generally counterproductive because additional heterotopic ossification develops at the operative site. Rarely, a joint may be repositioned surgically to improve the patient's overall functional status. Spinal bracing is ineffective, and surgical intervention is associated with numerous complications [88].

The ultimate goal of FOP research is the development of treatments that will prevent, halt, or even reverse the progression of the condition. The prevention and treatment of heterotopic ossification in FOP, as in any of the more common forms of heterotopic ossification, will ultimately be based on at least one of four principles: disrupting the relevant inductive signaling pathways, blocking the immunological and/or inflammatory triggers, suppressing the relevant osteoprogenitor cells in the target tissues, and modifying the tissue environment conducive to heterotopic osteogenesis (Fig. 4).

The identification of the recurrent heterozygous missense point mutation that causes FOP in all classically affected individuals provides a specific druggable target and a rational point of intervention in a critical signaling pathway [89]. Plausible therapeutic approaches to inhibiting BMP signaling in FOP include inhibitory RNA technology, monoclonal antibodies directed against ACVR1/ALK2, and orally available small molecule selective signal transduction inhibitors (STIs) of ACVR1/ALK2 [89], such as Dorsomorphin or its derivatives [90] which inhibit hetero-

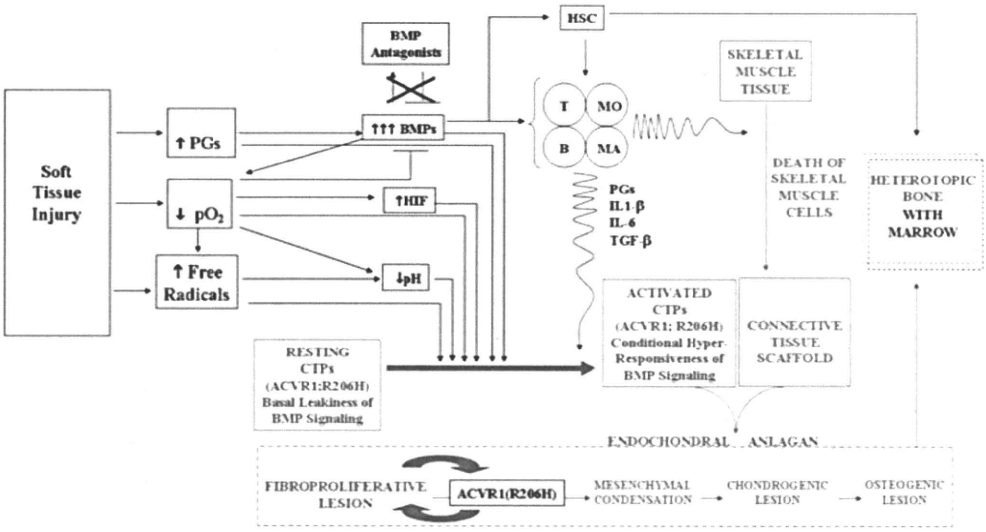

Fig. 4. Hypothetical model of skeletal metamorphosis in fibrodysplasia ossificans progressiva. (Adapted from Kaplan FS, et al. (2007) Morphogen receptor genes and metamorphogens: skeleton keys to the metamorphosis. Ann N Y Acad Sci 1116:113–133 [38].) *PGs*, prostaglandins; pO_2, tissue oxygen tension; *HIF*, hypoxia-inducing factor; *CTPs*, connective tissue progenitor cells; *HSC*, hematopoietic stem cells; *T*, T cells; *B*, B cells; *Mo*, monocytes; *MA*, mast cells; *IL-1β*, interleukin-1β; *IL-6*, interleukin 6; *TGF-β*, transforming growth factor-beta; *straight arrows*, progression; *wavy arrows*, influence

topic ossification in inflammation-induced models of BMP pathway overactivity.

Metamorphosis and tissue engineering: harnessing the FOP metamorphogene

ACVR1/ALK2 (R206H) is the first identified human metamorphogene and provides a genetic basis for understanding the general biological principles that orchestrate the pathological transformation of one normal organ system into another. The immediate goal of FOP research is to understand the molecular and cellular pathophysiology of this process and to use that knowledge to develop pharmacological methods to block and prevent it. Conversely, it may be possible to harness the gene mutation that causes FOP to create new skeletal elements in a controlled way—for patients who have osteoporotic fractures, for those with severe bone loss from trauma or neoplasms, for those with fractures that fail to heal, for those with spinal fusions that are slow to heal, or for those with congenital malformations of the spine and limbs who need new bone. The discovery of the FOP gene, the first human metamorphogene, is a monumental milestone in an epic journey to understand the biological principles that regulate tissue stability and skeletal metamorphosis following embryogenesis.

Other examples of skeletal metamorphosis in humans

Classic FOP and its phenotypic and genotypic variants are the most dramatic, but not the only, examples of skeletal metamorphosis in humans. There are, for example, many acquired forms of skeletal metamorphosis that are spatially and temporally limited. Some are fairly common, such as the heterotopic ossification that occurs following closed head injury, spinal cord injury, total hip replacement, athletic injury, and blast injuries from war [18,91–94]. Additionally, approximately 13% of individuals with end-stage valvular heart disease form mature heterotopic bone in the aortic valve [91]. To date, there are few studies on the molecular pathogenesis in these more common disorders of skeletal metamorphosis. Where it has been examined, the histology is similar in all, and it would not be surprising if the ACVR1/ALK2 signaling pathway was involved in the more common as in the rarer forms of skeletal metamorphosis.

Nature does not use different genes, molecules, and pathways for common conditions or for rare ones. Rather, it is often the rare disease that reveals which gene, molecule, or pathway nature hijacks in its common infirmities [95]. William Harvey, the discoverer of the circulatory system, wrote in 1657:

> "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." [96]

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References

1. Kaplan FS, Glaser DL, Pignolo RJ, Shore EM (2005) Introduction. Clin Rev Bone Miner Metab 3(3–4):175–177
2. Kaplan FS, Glaser DL, Shore EM, Deirmengian GK, Gupta R, Delai P, Morhart R, Smith R, Le Merrer M, Rogers JG, Connor JM, Kitterman JA (2005) The phenotype of fibrodysplasia ossificans progressiva. Clin Rev Bone Miner Metab 3:183–188
3. Shore EM, Xu M, Feldman GJ, Fenstermacher DA, Cho T-J, Choi IH, Connor JM, Delai P, Glaser DL, Le Merrer M, Morhart R, Rogers JG, Smith R, Triffitt JT, Urtizberea JA, Zasloff M, Brown MA, Kaplan FS (2006) A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. Nat Genet 38:525–527
4. Cohen RB, Hahn GV, Tabas J, Peeper J, Levitz CL, Sando A, Sando N, Zasloff M, Kaplan FS (1993) The natural history of heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. J Bone Joint Surg Am 75:215–219
5. Rocke DM, Zasloff M, Peeper J, Cohen RB, Kaplan FS (1994) Age and joint-specific risk of initial heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. Clin Orthop Relat Res 301:243–248
6. Kaplan FS, Tabas J, Gannon FH, Finkel G, Hahn GV, Zasloff MA (1993) The histopathology of fibrodysplasia ossificans progressiva: an endochondral process. J Bone Joint Surg Am 75:220–230
7. Gannon FH, Valentine BA, Shore EM, Zasloff MA, Kaplan FS (1998) Acute lymphocytic infiltration in an extremely early lesion of fibrodysplasia ossificans progressiva. Clin Orthop Relat Res 346:19–25
8. Glaser DL, Economides AN, Wang L, Liu X, Kimble RD, Fandl JP, Wilson JM, Stahl N, Kaplan FS, Shore EM (2003) In vivo somatic cell gene transfer of an engineered noggin mutein prevents BMP4-induced heterotopic ossification. J Bone Joint Surg Am 85:2332–2342
9. Pignolo RJ, Suda RK, Kaplan FS (2005) The fibrodysplasia ossificans progressiva lesion. Clin Rev Bone Miner Metab 3:195–200
10. Lanchoney TF, Cohen RB, Rocke DM, Zasloff MA, Kaplan FS (1995) Permanent heterotopic ossification at the injection site after diphtheria-tetanus-pertussis immunizations in children who have fibrodysplasia ossificans progressiva. J Pediatr 126:762–764
11. Janoff HB, Zasloff MA, Kaplan FS (1996) Submandibular swelling in patients with fibrodysplasia ossificans progressiva. Otolaryngol Head Neck Surg 114:599–604
12. Luchetti W, Cohen RB, Hahn GV, Rocke DM, Helpin M, Zasloff M, Kaplan FS (1996) Severe restriction in jaw movement after routine injection of local anesthetic in patients who have fibrodysplasia ossificans progressiva. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 81:21–25
13. Glaser DL, Rocke DM, Kaplan FS (1998) Catastrophic falls in patients who have fibrodysplasia ossificans progressiva. Clin Orthop Relat Res 346:110–116
14. Scarlett RF, Rocke DM, Kantanie S, Patel JB, Shore EM, Kaplan FS (2004) Influenza-like viral illnesses and flare-ups of fibrodysplasia ossificans progressiva. Clin Orthop Relat Res 423:275–279
15. Connor JM, Evans DA (1982) Fibrodysplasia ossificans progressiva. The clinical features and natural history of 34 patients. J Bone Joint Surg Br 64:76–83
16. Smith R (1988) Fibrodysplasia (myositis) ossificans progressiva: clinical lessons from a rare disease. Clin Orthop Relat Res 346:7–14
17. Kaplan FS, Shore EM, Connor JM (2002) Fibrodysplasia ossificans progressiva (FOP). In: Royce PM, Steinmann B (eds) Connective Tissue and Its Heritable Disorders: Molecular, Genetic, and Medical Aspects, 2nd edn. Wiley-Liss, New York, pp 827–840
18. Kaplan FS, Glaser DL, Hebela N, Shore EM (2004) Heterotopic ossification. J Am Acad Orthop Surg 12:116–125
19. Kaplan FS, Glaser DL, Shore EM (2006) Fibrodysplasia (myositis) ossificans progressiva. In: Favus MJ (ed). Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 6th edn. The American Society for Bone and Mineral Research, Washington, DC, pp 450–453
20. Moriatis JM, Gannon FH, Shore EM, Bilker W, Zasloff MA, Kaplan FS (1997) Limb swelling in patients who have fibrodysplasia ossificans progressiva. Clin Orthop Relat Res 336:247–253
21. Kaplan FS, Glaser DL (2005) Thoracic insufficiency syndrome in patients with fibrodysplasia ossificans progressiva. Clin Rev Bone Miner Metab 3:213–216
22. Shore EM, Feldman GJ, Xu M, Kaplan FS (2005) The genetics of fibrodysplasia ossificans progressiva. Clin Rev Bone Miner Metab 3:201–204
23. Kitterman JA, Kantanie S, Rocke DM, Kaplan FS (2005) Iatrogenic harm caused by diagnostic errors in fibrodysplasia ossificans progressiva. Pediatrics 116:654–661
24. Kaplan FS, Xu M, Glaser DL, Collins F, Connor M, Kitterman J, Sillence D, Zackai E, Ravitsky V, Zasloff M, Ganguly A, Shore E (2008) Early diagnosis of fibrodysplasia ossificans progressiva (FOP). Pediatrics 121:e1295–1300
25. Schaffer AA, Kaplan FS, Tracy MR, O'Brien ML, Dormans JP, Shore EM, Harland RM, Kusumi K (2005) Developmental anomalies of the cervical spine in patients with fibrodysplasia ossificans progressiva are distinctly different from those in patients with Klippel–Feil syndrome. Spine 30:1379–1385
26. Groppe J, Greenwald J, Wiater E, Rodriguez-Leon J, Economides A, Kwaitowski W, Affolter M, Vale WW, Izpisua-Belmonte JC, Choe S (2002) Structural basis of BMP signalling inhibition by Noggin, a novel cystine knot protein. Nature (Lond) 420:636–642
27. Lucotte G, Semonin O, Lutz P (1999) A de novo heterozygous deletion of 42 base-pairs in the noggin gene of a fibrodysplasia ossificans progressiva. Clin Genet 56:469–470
28. Semonin O, Fontaine K, Daviaud C, Ayuso C, Lucotte G (2001) Identification of three novel mutations of the noggin gene in patients with fibrodysplasia ossificans progressiva. Am J Med Genet 102:314–317
29. Lucotte G, La Garde JP, and members of the FOP European Research Group (2007) Mutations of the noggin and of the activin A type I receptor genes in fibrodysplasia ossificans progressiva (FOP). Genet Couns 18:349–352
30. Xu M-Q, Feldman G, Le Merrer M, Shugart YY, Glaser DL, Urtizberea JA, Fardeau M, Connor JM, Triffitt J, Smith R, Shore EM, Kaplan FS (2000) Linkage exclusion and mutational analysis of the noggin gene in patients with fibrodysplasia ossificans progressiva. Clin Genet 58:291–298
31. Cohen MM Jr (2002) Bone morphogenetic proteins with some comments on fibrodysplasia ossificans progressiva and noggin. Am J Med Genet 109:87–92
32. Carey JC (2002) Editor's note. Am J Med Genet 109:160
33. Xu M, Shore EM, Kaplan FS (2002) Reported noggin mutations are PCR errors. Am J Med Genet 109:161
34. Warman ML (2002) Significant difference of opinion regarding the role of noggin in fibrodysplasia ossificans progressiva. Am J Med Genet 109:162
35. Deirmengian GK, Hebela NM, O'Connell M, Glaser DL, Shore EM, Kaplan FS (2008) Proximal tibial osteochondromas in patients with fibrodysplasia ossificans progressiva (FOP). J Bone Joint Surg Am 90:366–374
36. Kaplan FS, McCluskey W, Hahn G, Tabas J, Muenke M, Zasloff MA (1993) Genetic transmission of fibrodysplasia ossificans progressiva. J Bone Joint Surg Am 75:1214–1220
37. Hebela N, Shore EM, Kaplan FS (2005) Three pairs of monozygotic twins with fibrodysplasia ossificans progressiva: the role of environment in the progression of heterotopic ossification. Clin Rev Bone Miner Metab 3:205–208
38. Kaplan FS, Groppe J, Pignolo RJ, Shore EM (2007) Morphogen receptor genes and metamorphogenes: skeleton keys to the metamorphosis. Ann N Y Acad Sci 1116:113–133
39. Urist MR (1965) Bone formation by autoinduction. Science 150:893–899
40. Urist MR, Nakagawa M, Nakata N, Nogami H (1978) Experimental, myositis ossificans: cartilage and bone formation in muscle in response to a diffusable bone matrix-derived morphogen. Arch Pathol Lab Med 102:312–316
41. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA (1988) Novel regulators of bone formation: molecular clones and activities. Science 242:1528–1534
42. Gannon FH, Glaser D, Caron R, Thompson LD, Shore EM, Kaplan FS (2001) Mast cell involvement in fibrodysplasia ossificans progressiva. Hum Pathol 32:842–848

43. Kan L, Hu M, Gomes WA, Kessler JA (2004) Transgenic mice overexpressing BMP4 develop a fibrodysplasia ossificans progressiva (FOP)-like phenotype. Am J Pathol 165:1107–1115
44. Kan L (2005) The neuron-specific enolase-bone morphogenetic protein 4 transgenic mouse. Clin Rev Bone Miner Metab 3: 235–237
45. Katagiri T, Yamaguchi A, Komaki M, Abe E, Takahashi N, Ikeda T, Rosen V, Wozney JM, Fujisawa-Sehara A, Suda T (1994) Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. J Cell Biol 127: 1755–1766
46. Akiyama S, Katagiri T, Namiki M, Yamaji N, Yamamoto N, Miyama K, Shibuya H, Ueno N, Wozney JM, Suda T (1997) Constitutively active BMP type I receptors transduce BMP2 signals without the ligand in C2C12 myoblasts. Exp Cell Res 235:362–369
47. Massagué J (2000) How cells read TGF-β signals. Nat Rev Mol Cell Biol 1:169–178
48. Hoffman A, Gross G (2001) BMP signaling pathways in cartilage and bone formation. Crit Rev Eukaryot Gene Expr 11:23–45
49. ten Dijke P, Korchynskyi O, Valdimarsdottir G, Goumans MJ (2003) Controlling cell fate by bone morphogenetic protein receptors. Mol Cell Endocrinol 211:105–113
50. Kloen P, Di Paola M, Borens O, Richmond J, Perino G, Helfet DL, Goumans MJ (2003) BMP signaling components are expressed in human fracture callus. Bone (NY) 33:362–371
51. Gazzerro E, Canalis E (2006) Bone morphogenetic proteins and their antagonists. Rev Endocr Metab Disord 7:51–65
52. Shi Y, Massagué J (2003) Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell 113:685–700
53. Massagué J, Seoane J, Wotton D (2005) Smad transcription factors. Genes Dev 19:2783–2810
54. Schmierer B, Hill CS (2007) TGFβ-SMAD signal transduction: molecular specificity and functional flexibility. Mol Cell Biol 8:970–982
55. Lander AD (2007) Morpheus unbound: reimagining the morphogen gradient. Cell 128:245–256
56. Kaplan FS, Tabas JA, Zasloff MA (1990) Fibrodysplasia ossificans progressiva (FOP): a clue from the fly? Calcif Tissue Int 47: 117–125
57. Shafritz AB, Shore EM, Gannon FH, Zasloff MA, Taub R, Muenke M, Kaplan FS (1996) Over-expression of an osteogenic morphogen in fibrodysplasia ossificans progressiva. N Engl J Med 335: 555–561
58. Gannon FH, Kaplan FS, Olmsted E, Finkel G, Zasloff MA, Shore EM (1997) Bone morphogenetic protein 2/4 in early fibromatous lesions of fibrodysplasia ossificans progressiva. Hum Pathol 28: 339–343
59. Ahn J, Serrano de la Peña L, Shore EM, Kaplan FS (2003) Paresis of a bone morphogenetic protein-antagonist response in a genetic disorder of heterotopic skeletogenesis. J Bone Joint Surg Am 85:667–674
60. Serrano de la Peña L, Billings PC, Fiori JL, Ahn J, Shore EM, Kaplan FS (2005) Fibrodysplasia ossificans progressiva, a disorder of ectopic osteogenesis, misregulates cell surface expression and trafficking of BMPRIA. J Bone Miner Res 20:1168–1176
61. Fiori JL, Billings PC, Serrano de la Peña L, Kaplan FS, Shore EM (2006) Dysregulation of the BMP-p38 MAPK signaling pathway in cells from patients with fibrodysplasia ossificans progressiva. J Bone Miner Res 21:902–909
62. Kaplan FS, Fiori J, Serrano de la Peña L, Ahn J, Billings PC, Shore EM (2006) Dysregulation of the BMP4 signaling pathway in fibrodysplasia ossificans progressiva. Ann N Y Acad Sci 1068: 54–65
63. Billings PC, Fiori JL, Bentwood JL, O'Connell MP, Jiao X, Nussbaum B, Caron RJ, Shore EM, Kaplan FS (2008) Dysregulated BMP4 signaling and enhanced osteogenic differentiation of connective tissue progenitor cells from patients with fibrodysplasia ossificans progressiva. J Bone Miner Res 23:305–313
64. Harradine KA, Akhurst RJ (2006) Mutations of TGF-β signaling molecules in human disease. Ann Med 38:403–414
65. Zhang D, Schwarz EM, Rosier RN, Zuscik MJ, Puzas JE, O'Keefe RJ (2003) Alk2 functions as a BMP type I receptor and induces Indian hedgehog in chondrocytes during skeletal development. J Bone Miner Res 18:1593–1604
66. Fukada T, Kohda M, Kanomata K, Nojima J, Kamizono J, Oda H, Nakayama K, Ohtake A, Miyazono K, Jimi E, Owan I, Okazaki Y, Katagiri T (2007) A constitutively activated BMP receptor, ALK2, induces heterotopic bone formation in patients with fibrodysplasia ossificans progressiva. J Bone Miner Res 22(suppl 1): S10
67. Shen Q, Xu M, Little SC, Kaplan FS, Mullins MC, Shore EM (2007) Activation of BMP signaling by the FOP ACVR1 R206H mutation. J Bone Miner Res 22(suppl 1):S43
68. Groppe JC, Shore EM, Kaplan FS (2007) Functional modeling of the ACVR1 (R206H) mutation in FOP. Clin Orthop Relat Res 462:87–92
69. Wang T, Li B-Y, Danielson PD, Shah PC, Rockwell S, Lechleider RJ, Martin J, Manganaro T, Donahoe PK (1996) The immunophilin FKBP12 functions as a common inhibitor of the TGF-β family type I receptors. Cell 86:435–444
70. Chen Y-G, Liu F, Massagué J (1997) Mechanism of TGF-β receptor inhibition by FKBP12. EMBO J 16:3866–3876
71. Huse M, Chen YG, Massagué J, Kuriyan J (1999) Crystal structure of the cytoplasmic domain of the type I TGF-β receptor complex with FKBP12. Cell 96:425–436
72. Huse M, Muir TW, Xu L, Chen YG, Kuriyan J, Massagué J (2001) The TGF-β receptor activation process: an inhibitor-to-substrate binding switch. Mol Cell 8:671–682
73. Ebisawa T, Fukuchi M, Murakami G, Chiba T, Tanaka K, Imamura T, Miyazono K (2001) Smurf1 interacts with transforming growth factor-β type 1 receptor through Smad 7 and induces receptor degradation. J Biol Chem 276:12477–12480
74. Yamaguchi T, Kurisaki A, Yamakawa N, Minakuchi K, Sugino H (2006) FKBP12 functions as an adaptor of the Smad7-Smurf1 complex on activin type I receptor. J Mol Endocrinol 36:569–579
75. Kaplan FS, Shore EM, Gupta R, Billings PC, Glaser DL, Pignolo RJ, Graf D, Kamoun M (2005) Immunological features of fibrodysplasia ossificans progressiva and the dysregulated BMP4 pathway. Clin Rev Bone Miner Metab 3:189–193
76. Olmsted-Davis E, Gannon FH, Ozen M, Ittmann MM, Gugala Z, Hipp JA, Moran KM, Fouletier-Dilling CM, Schumara-Martin S, Lindsey RW, Heggeness MH, Brenner MK, Davis AR (2007) Hypoxic adipocytes pattern early heterotopic bone formation. Am J Pathol 170:620–632
77. Kaplan FS, Le Merrer M, Glaser DL, Pignolo RJ, Goldsby R, Kitterman JA, Groppe J, Shore EM (2008) Fibrodysplasia ossificans progressiva. Best Pract Res Clin Rheumatol 22:191–205
78. Hakim M, Hage W, Lovering RM, Moorman CT III, Curl LA, De Deyne PG (2005) Dexamethasone and recovery of contractile tension after a muscle injury. Clin Orthop Relat Res 439:235–242
79. Järvinen TA, Järvinen TL, Kääriäinen M, Kalimo H, Järvinen M (2005) Muscle injuries: biology and treatment. Am J Sports Med 33:745–764
80. Wynn TA (2007) Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 117:524–529
81. Vanden Bossche LC, Van Maele G, Wojtowicz I, De Cock K, Vertriest S, De Muynck M, Rimbaut S, Vanderstraeten GG (2007) Free radical scavengers are more effective than indomethacin in the prevention of experimentally induced heterotopic ossification. J Orthop Res 25:267–272
82. Schipani E, Ryan HE, Didrickson S, Kobayashi T, Knight M, Johnson RS (2001) Hypoxia in cartilage: HIF-1 alpha is essential for chondrocyte growth arrest and survival. Genes Dev 15: 2865–2876
83. Pfander D, Cramer T, Schipani E, Johnson RS (2003) HIF-1-alpha controls extracellular matrix synthesis by epiphyseal chondrocytes. J Cell Sci 116:1819–1826
84. Provot S, Ziny KD, Gunes Y, Kathri R, Le Q, Kronenberg HM, Johnson RS, Longaker MT, Giaccia AJ, Schipani E (2007) Hif-1-alpha regulates differentiation of limb bud mesenchyme and joint development. J Cell Biol 177:451–464
85. Kaplan FS, Glaser DL, Shore EM, Pignolo RJ, Xu M, Zhang Y, Senitzer D, Forman SJ, Emerson SG (2007) Hematopoietic stem-cell contribution to ectopic skeletogenesis. J Bone Joint Surg Am 89:347–357
86. Stoick-Cooper CL (2007) Advances in signaling in vertebrate regeneration as a prelude to regenerative medicine. Genes Dev 21:1292–1315

87. Glaser DL, Kaplan FS (2005) Treatment considerations for the management of fibrodysplasia ossificans progressiva. Clin Rev Bone Miner Metab 3:243–250
88. Shah PB, Zasloff MA, Drummond D, Kaplan FS (1994) Spinal deformity in patients who have fibrodysplasia ossificans progressiva. J Bone Joint Surg Am 76:1442–1450
89. Kaplan FS, Glaser DL, Pignolo RJ, Shore EM (2007) A new era for fibrodysplasia ossificans progressiva: a druggable target for the second skeleton. Expert Opin Biol Ther 7:705–712
90. Yu PB, Hong CC, Sachidanandan C, Babitt JL, Deng DY, Hoyng SA, Lin HY, Bloch KD, Peterson RT (2008) Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. Nat Chem Biol 4:33–41
91. Mohler ER III, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS (2001) Bone formation and inflammation in cardiac valves. Circulation 20:1522–1528
92. Pignolo RJ, Foley KL (2005) Nonhereditary heterotopic ossification: implications for injury, arthropathy, and aging. Clin Rev Bone Miner Metab 3:261–266
93. Wolf JM (2006) The genetic key to a rare disease and its impact on orthopedics. Orthopedics 29:672
94. Potter BK, Burns TC, Lacap AP, Granville RR, Gajkewski D (2006) Heterotopic ossification in the residual limbs of traumatic and combat-related amputees. J Am Acad Orthop Surg 14: S191–S197
95. Kaplan FS (2006) The key to the closet is the key to the kingdom: a common lesson of rare diseases. Orphan Disease Update 24:1–9
96. Willis R (1989) The Works of William Harvey. University of Pennsylvania Press, Philadelphia, pp 616–617

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BMP type I receptor inhibition reduces heterotopic ossification

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Fibrodysplasia ossificans progressiva (FOP) is a congenital disorder of progressive and widespread postnatal ossification of soft tissues[1–4] and is without known effective treatments. Affected individuals harbor conserved mutations in the *ACVR1* gene that are thought to cause constitutive activation of the bone morphogenetic protein (BMP) type I receptor, activin receptor-like kinase-2 (ALK2)[5]. Here we show that intramuscular expression in the mouse of an inducible transgene encoding constitutively active ALK2 (caALK2), resulting from a glutamine to aspartic acid change at amino acid position 207, leads to ectopic endochondral bone formation, joint fusion and functional impairment, thus phenocopying key aspects of human FOP. A selective inhibitor of BMP type I receptor kinases, LDN-193189 (ref. 6), inhibits activation of the BMP signaling effectors SMAD1, SMAD5 and SMAD8 in tissues expressing caALK2 induced by adenovirus specifying Cre (Ad.Cre). This treatment resulted in a reduction in ectopic ossification and functional impairment. In contrast to localized induction of caALK2 by Ad.Cre (which entails inflammation), global postnatal expression of caALK2 (induced without the use of Ad.Cre and thus without inflammation) does not lead to ectopic ossification. However, if in this context an inflammatory stimulus was provided with a control adenovirus, ectopic bone formation was induced. Like LDN-193189, corticosteroid treatment inhibits ossification in Ad.Cre-injected mutant mice, suggesting caALK2 expression and an inflammatory milieu are both required for the development of ectopic ossification in this model. These results support the role of dysregulated ALK2 kinase activity in the pathogenesis of FOP and suggest that small molecule inhibition of BMP type I receptor activity may be useful in treating FOP and heterotopic ossification syndromes associated with excessive BMP signaling.

Individuals with FOP typically present within the first decade of life with progressive ectopic calcification of muscles and connective tissues after physical trauma, surgery, viral illness or myositis[1–4]. FOP results in severe debilitation and reduced life expectancy due to joint fusion and restrictive lung disease with thoracic involvement. A recent linkage and sequencing analysis identified heterozygous mutations in *ACVR1*, the gene encoding the BMP type I receptor ALK2, in all affected members from seven families[5,7]. BMP ligands facilitate the phosphorylation and activation of BMP type I receptors (ALK2, ALK3 and ALK6) by BMP type II receptors (BMPRII, ActRIIA and ActRIIB). Activated BMP type I receptors phosphorylate BMP-responsive SMAD1, SMAD5 and SMAD8, which translocate to the nucleus to regulate the transcription of genes, including the Inhibitor of DNA binding (*ID*) gene family, with broad effects on growth and differentiation[8]. The classic FOP-associated ALK2 mutation, R206H, is predicted to disrupt an α-helix in the glycine-serine regulatory domain and alter local electrostatic potential to perturb intra- or intermolecular interactions required for kinase regulation[5,9], rendering ALK2 constitutively active. Heterozygous mutations affecting the adjacent residue, Q207E, have been identified in phenotypic variant FOP[10]. The Q207E mutation and a well described man-made ALK2 mutation affecting the same residue, Q207D[11], may exert similarly disruptive effects on the glycine-serine domain structure and to cause constitutive activation of ALK2. *In vivo*, transfer of the gene encoding $ALK2^{Q207D}$ but not wild-type ALK2 induces chondrogenic differentiation in chick embryos and promotes endochondral bone growth in cortical allografts[12,13], consistent with potent osteogenic effects of constitutively activating ALK2 mutations.

To further explore the ability of constitutively active ALK2 to induce ectopic calcification, we took advantage of the availability of transgenic mice expressing an inducible $ALK2^{Q207D}$

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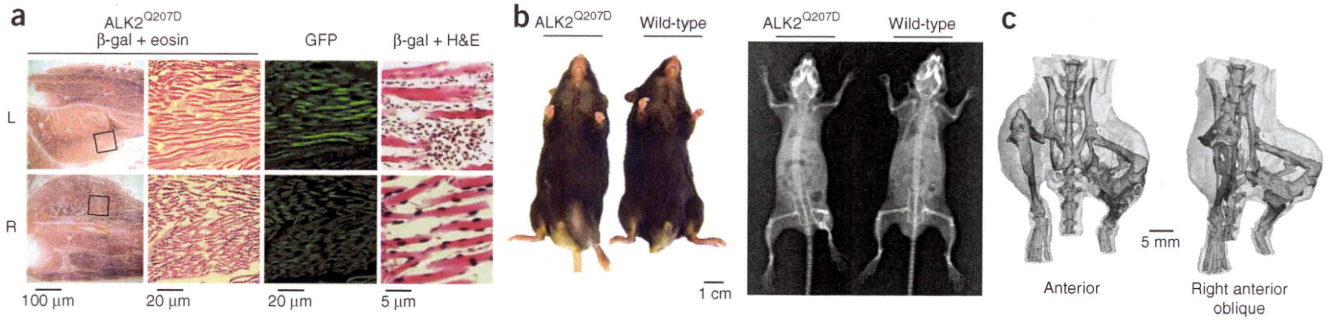

Figure 1 Mouse model of FOP. (**a**) Staining of muscle sections from Ad.Cre-injected conditional caALK2–expressing (ALK^{Q207D}) mice. The injection done at P7 induced recombination in myocytes of the left (L) gastrocnemius and soleus muscles at P11, as evidenced by loss of nuclear β-galactosidase (β-gal) staining and gain of GFP expression. Squares indicate areas of low magnification examined at higher magnification to the right. H&E staining reveals mononuclear cell infiltrates and myocyte edema in tissues undergoing recombination, but not in uninjected right (R) hindlimb muscles. (**b**) Left hindlimb postural abnormalities were observed grossly (left) at P30 in the Ad.Cre-injected left hindlimbs of conditional caALK2 mice, but not in Ad.Cre-injected wild-type mice. The X-ray image (right) shows Ad.Cre-induced ectopic calcifications involving the left gastrocnemius, soleus and hamstring muscles of conditional caALK2 mice at P30. (**c**) Three-dimensional reconstructed images from μCT cross-sections of an Ad.Cre-injected, conditional caALK2–expressing mouse on P30 showing intramuscular ectopic bone within the left gastrocnemius, soleus, tibialis and hamstring muscles (rendered in light gray) fusing with the pelvis and proximal femur.

(CAG-Z-EGFP-caALK2, or conditional caALK2). When the transgene is globally expressed during embryogenesis, mice are arrested at mid-gestation[14], in contrast to individuals with FOP, who appear essentially normal at birth except for shortened great toes[2]. To circumvent the embryonic lethality of this transgene, we induced postnatal over-expression of $ALK2^{Q207D}$ in the left hindlimbs of mice with retro-popliteal injection of Ad.Cre (1×10^8 plaque-forming units (PFU)) on postnatal day 7 (P7). High-frequency, Cre-mediated recombination was evident by P11, with loss of nuclear β-galactosidase staining and gain of cytoplasmic GFP expression in myocytes, ligaments and vasculature only in the injected area (**Fig. 1a**). Mononuclear infiltrates and myofiber edema were apparent in the left gastrocnemius, soleus and hamstring muscles, consistent with myositis induced by intra-muscular adenovirus injection[15], whereas muscles of uninjected limbs appeared normal (**Fig. 1a**).

Conditional caALK2–expressing mice injected with Ad.Cre in the left hindlimb developed severely decreased mobility in the injected limb by P30, with loss of passive flexion in hip, knee and ankle joints when examined under anesthesia, whereas wild-type mice injected with Ad.Cre retained normal posture and range of motion (**Fig. 1b**). By radiography and micro-computed tomography (μCT), bony calluses were evident in injected hindlimbs of conditional caALK2–expressing mice, circumferentially encasing the tibia and fibula (**Fig. 1b,c**). These calluses frequently fused with the pelvis and femur, rendering hip, knee and ankle joints immobile and extended and preventing the use of those limbs during locomotion (**Supplementary Videos 1** and **2** online). At P30, the penetrance of heterotopic ossification and immobility associated with local induction of caALK2 by this technique was 100% (data not shown).

We recently identified a small-molecule inhibitor of BMP type I receptors, dorsomorphin (**Fig. 2a**), which selectively blocks ALK2, ALK3 and ALK6 activity[16]. We subsequently described the synthesis of potent and specific derivatives of dorsomorphin[6]. By reiteratively testing modifications of the parent molecule, we found that replacement of the pendent pyridine ring with a 4-quinolinyl group could improve potency that replacement of the 2-(1-piperidinyl)ethoxy group with piperazine improved metabolic stability and that, in general, modifications of the pyrazolo[1,5-a]pyrimidine core were not tolerated[6]. An optimized molecule, LDN-193189 (**Fig. 2a**), inhibited BMP4-mediated Smad1, Smad5 and Smad8 activation with greater potency than did dorsomorphin (half-maximal inhibitory concentration (IC_{50}) = 5 nM versus 470 nM) while retaining 200-fold selectivity for BMP signaling versus transforming growth factor-β (TGF-β) signaling (IC_{50} for TGF-β ≥ 1,000 nM; **Fig. 2a–c**). LDN-193189 efficiently inhibited transcriptional activity of the BMP type I receptors ALK2 and ALK3 (IC_{50} = 5 nM and 30 nM, respectively), with substantially weaker effects on activin and the TGF-β type I receptors ALK4, ALK5 and ALK7 (IC_{50} ≥ 500 nM, **Supplementary Fig. 1** online) and increased selectivity for BMP signaling versus AMP-activated protein kinase, platelet-derived growth factor receptor and mitogen–activated protein kinase signaling pathways as compared to the parent compound[16] (**Supplementary Fig. 2** online). LDN-193189 blocked the transcriptional activity induced by either constitutively active $ALK2^{R206H}$ or $ALK2^{Q207D}$ mutant proteins (**Fig. 2d,e**). These findings suggest that LDN-193189 might affect BMP-induced osteoblast differentiation. In fact, LDN-193189 inhibited the induction of alkaline phosphatase activity in C2C12 cells by BMP4 even when administered 12 h after BMP stimulation (**Fig. 2f**), indicating sustained BMP signaling activity is needed for osteogenic differentiation, as we have previously observed in vascular smooth muscle cells[17].

To assess the impact of $ALK2^{Q207D}$ on BMP signaling, we isolated and cultured pulmonary artery smooth muscle cells (PASMCs) from conditional caALK2–expressing mice. Baseline phosphorylation of Smad1, Smad5 and Smad8 was increased in cells infected with Ad.Cre compared to those infected with Ad.GFP (**Fig. 2g**), consistent with increased basal BMP signaling after Cre-mediated induction of $ALK2^{Q207D}$. PASMCs expressing $ALK2^{Q207D}$ also showed hyperresponsiveness to BMP ligands, consistent with observations in cells that express the mutant $ALK2^{R206H}$ protein[18,19]. Enhanced Smad1, Smad5 and Smad8 activation in cells expressing $ALK2^{Q207D}$ was effectively inhibited by treatment with LDN-193189 (**Fig. 2g**).

To determine the pharmacokinetics of LDN-193189, we measured its plasma concentration after administration of a single dose (3 mg kg^{-1} intraperitoneally (i.p.)) in C57BL/6 mice. LDN-193189 remained at levels several fold higher than its *in vitro* IC_{50} for >8 h (**Supplementary Fig. 3** online), suggesting sustained inhibition of BMP signaling might be obtained by bolus dosing. To assess the effect of ALK2 kinase inhibition on ectopic calcification *in vivo*, we injected conditional caALK2–transgenic and wild-type mice with Ad.Cre on P7

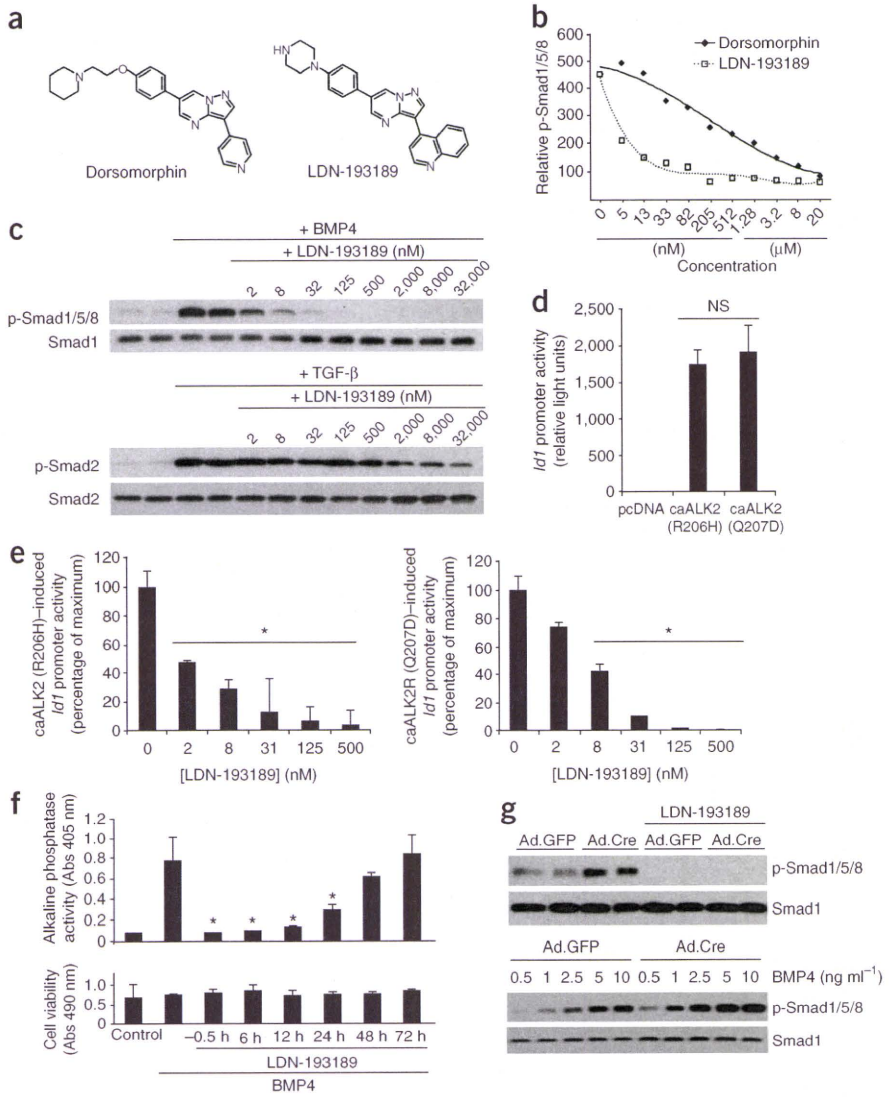

Figure 2 Effect of LDN-193189 on BMP signaling and function. (**a**) Structures of dorsomorphin and LDN-193189. (**b**) Quantitative immunoblotting of PASMCs showing differential effects of dorsomorphin or LDN-193189 on BMP4–induced (10 ng ml^{-1}) phosphorylation of Smad1, Smad5 and Smad8 (p-Smad1/5/8), with IC_{50} values of ~400 nM and ~5 nM, respectively. (**c**) Immunoblot of PASMCs treated with LDN-193189 showing differential inhibition of BMP4 (10 ng ml^{-1}) or TGF-β (0.5 ng ml^{-1}) signaling (IC_{50} ~ 5 nM and ≥ 1 μM, respectively). (**d**) *Id1* promoter activity induced by transient transfection of COS cells with ALK2^{R206H} or ALK2^{Q207D}. *Id1* promoter activity (BRE-Luc) was increased by 250- to 300-fold over control plasmid (pcDNA, $n = 3$ measurements, mean ± s.d., NS, no significant difference). (**e**) Impact of LDN-193189 on the transcriptional activities of caALK2^{R206H} and caALK2^{Q207D} mutants, expressed as percentage of full *Id1* promoter activity ($n = 3$ measurements, mean ± s.d., $^*P < 0.05$ versus untreated). (**f**) Impact of LDN-193189 (100 nM) on BMP4-induced (10 ng ml^{-1}) osteoblast differentiation of C2C12 cells at various intervals before and after BMP4 treatment ($n = 6$ measurements, mean ± s.d., $^*P < 0.01$ versus BMP4 treatment alone) and on cell viability (bottom panel). (**g**) Top, immunoblot for p-Smad1/5/8 and total Smad1 in PASMCs expressing the conditional caALK2^{Q207D} transgene after infection with Ad.Cre or Ad.GFP showing the impact of pretreatment with LDN-193189 (100 nM). Bottom, immunoblot for phosphorylated Smad1/5/8 and total Smad1 in Ad.GFP- or Ad.Cre-infected PASMCs treated with BMP4 at varying concentrations.

and then treated them with LDN-193189 (3 mg kg^{-1} i.p. every 12 h) or with vehicle. Ad.Cre injection of conditional caALK2–transgenic mice led to mild calcifications surrounding the left tibia and fibula first visible at P13 on X-ray (**Fig. 3a**). By P15, these lesions were more prominent and extended to involve the distal femur (**Fig. 3a**). Lesions that effectively joined the hip, femur and tibia-fibula were present in all vehicle-treated, Ad.Cre-injected mice by P30, progressing to fusion of the left hip, knee and ankle joints by P60 (**Fig. 3a** and **Supplementary Fig. 4** online). Treatment of Ad.Cre-injected, caALK2-expressing mice with LDN-193189 prevented radiographic lesions at P15 in all mice examined (**Fig. 3a**). At P30, LDN-193189 prevented ectopic bone in approximately two-thirds of mice and attenuated lesions in the remainder, whereas at P60 LDN-193189 prevented ectopic bone in one-third of mice and attenuated lesions in the remainder (**Supplementary Fig. 4**). In contrast to vehicle-treated mice, LDN-193189–treated mice appeared to preserve knee and ankle joints at P30 and P60. Alizarin red and Alcian blue staining of vehicle-treated caALK2-expressing mice at P15 revealed ectopic bone encasing the tibia and fibula and increased cartilage formation in surrounding tissues (**Fig. 3b,c**). LDN-193189-treated mice showed no ectopic bone at P15 but did show enhanced cartilage formation in surrounding soft tissues compared to wild-type mice. Vehicle-treated, caALK2-expressing mice developed extensive ossification and fusion by P30 on μCT, whereas LDN-193189-treated, caALK2-expressing mice had small ectopic ossifications without joint fusion (**Fig. 3d**). Radiographic lesions in vehicle-treated mice correlated with abnormal hindlimb posture and decreased passive range of motion of hip, knee and ankle joints when the mice were examined under anesthesia, all of which were attenuated in LDN-193189-treated mice (**Fig. 3e**). A quantitative measure of passive range of motion in the ankle joint (**Fig. 3f**) appeared to be a sensitive marker of functional impairment (**Fig. 3g**), correlating with severe lesions found in vehicle-treated mutant mice while demonstrating significant rescue of function in LDN-193189–treated mice. In fact, LDN-193189–treated mice showed at least mildly impaired range of motion even in the absence of radiographically visible disease at P30, perhaps reflecting enhanced cartilage formation or early calcification (**Fig. 3a–c,g**). Consistent with this functional end point, vehicle-treated mice progressively lost use of the left hindlimb owing to joint fusion, whereas LDN-193189–treated mice retained use of the left hindlimb during ambulation at P15 and P30 (**Supplementary Videos 3–6** online). Treatment of wild-type or mutant mice with LDN-193189 under this regimen did not cause weight loss or growth retardation (**Supplementary Fig. 5** online), spontaneous fractures, decreased bone density (**Fig. 3a**) or lead to other skeletal, morphologic, hematological or behavioral abnormalities (data not shown).

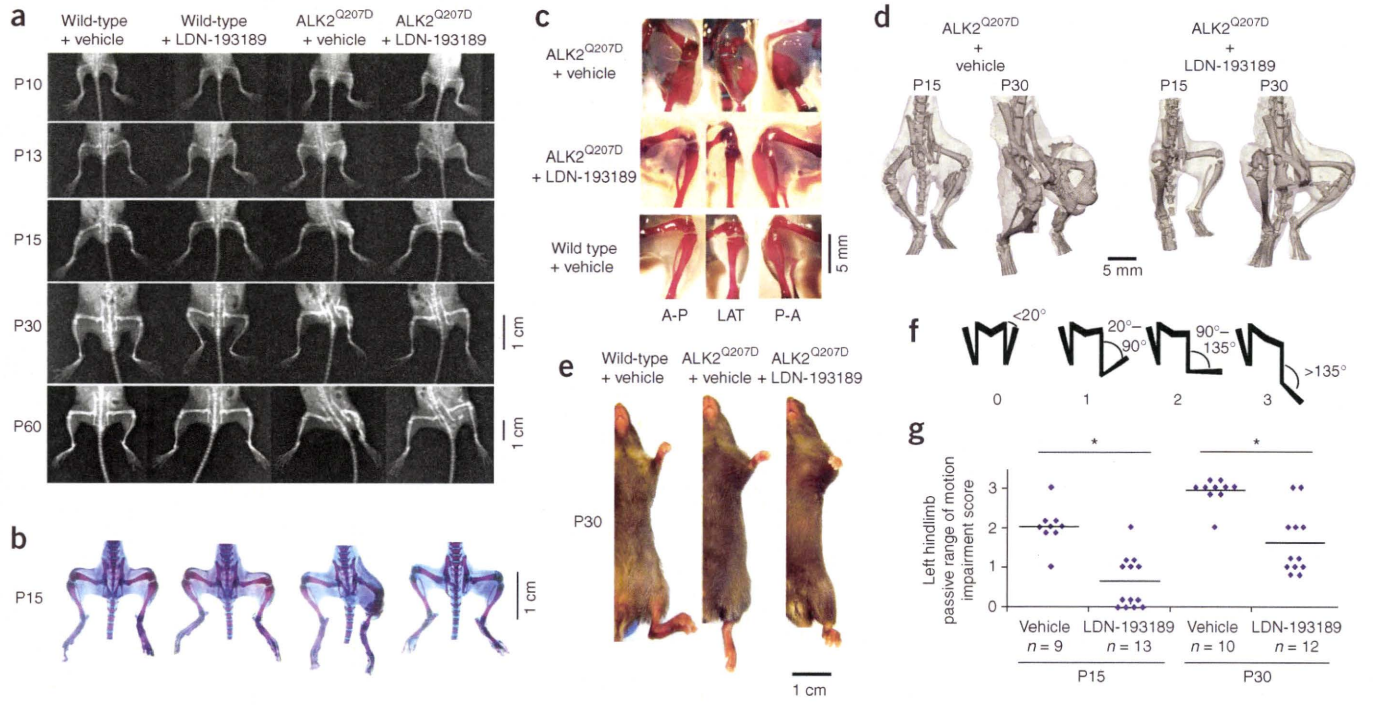

Figure 3 Impact of LDN-193189 on ectopic ossification *in vivo*. (**a**) Conditional caALK2–expressing mice receiving vehicle after Ad.Cre injection on P7 developed radiographic disease at P13, progressing to fusion of left hindlimb joints by P30–P60, whereas treatment with LDN-193189 diminished ectopic bone formation and preserved joint spaces over the same interval without inducing fractures, osteopenia or skeletal abnormalities (n = 3–5 mice per treatment group; data are representative of six independent experiments). (**b**,**c**) Alizarin red and Alcian blue staining of mice at P15 showing ectopic calcifications encasing the left tibia and fibula in vehicle-treated conditional caALK2–expressing mice, but not in LDN-193189–treated mice. Higher magnification images are shown in **c**. Ectopic bone or cartilage are absent in the wild-type hindlimb. A-P, anterior-posterior; LAT, lateral; P-A, posterior-anterior. (**d**) μCT imaging showing attenuated ectopic calcification in LDN-193189–treated mice as compared to vehicle-treated mice at P15 and P30. (**e**) Fixed extension of left hip, knee and ankle joints, as evident in anesthetized and flaccid Ad.Cre-injected conditional caALK2–mutant mice at P30. The extension is attenuated in LDN-193189-treated mice. (**f**) Passive range of motion impairment score, as assessed by the minimum angle formed by the ankle and tibia with passive dorsoflexion. (**g**) Impact of vehicle and LDN-193189 treatment upon passive range of motion impairment in Ad.Cre-injected conditional caALK2–mutant mice at P15 and P30 (n as indicated, bars represent mean, $^*P < 0.001$).

Vehicle-treated, Ad.Cre-injected, caALK2-expressing mice showed increased amounts and nuclear accumulation of phosphorylated Smad1, Smad5 and Smad8 in the left gastrocnemius, soleus and hamstring muscles (**Fig. 4a**), whereas the uninjected right gastrocnemius had little detectable phosphorylated Smad1, Smad5 and Smad8. Consistent with enhanced Smad1, Smad5 and Smad8 signaling, *Id1* expression was more than threefold greater in the hindlimb muscles of Ad.Cre-injected, caALK2-expressing mice than in wild-type or uninjected mutant controls (**Supplementary Fig. 6** online). Within Ad.Cre-injected muscle tissues, but not controls, a subset of myocyte-like cells was observed to express the osteogenic runt-related transcription factor-2 (Runx2; **Fig. 4a**). Treatment of conditional caALK2–transgenic mice with LDN-193189 did not have an impact on recombination efficiency, myocyte edema or inflammation, but did result in diminished phosphorylated Smad1, Smad5 and Smad8 and Runx2 staining in the left gastrocnemius (**Fig. 4a**). By P15, maturing endochondral bone, marked by alkaline phosphatase–positive osteoblasts, chondrocyte-like cells and marrow cells were evident in Ad.Cre-injected muscles of conditional caALK2–expressing mice (**Fig. 4b**). The hindlimbs of Ad.Cre-injected, conditional caALK2–expressing mice treated with LDN-193189 developed substantially less endochondral bone but retained evidence of inflammation and myocyte injury (**Fig. 4b**). Histological evolution of lesions in affected mice thus paralleled that of human intramuscular FOP lesions, showing myocyte injury and inflammatory infiltrate followed by elaboration of a chondrogenic matrix, osteoblast-mediated mineralization and maturation into endochondral bone[20]. This process of ectopic ossification, beginning with activation of Smad1, Smad5 and Smad8, was markedly attenuated by LDN-193189 treatment.

To test the sufficiency of transgenic expression of caALK2 for the development of ectopic bone, we used an alternate recombination strategy. We achieved global postnatal expression of $ALK2^{Q207D}$ by mating conditional caALK2–transgenic mice with CAGGS-CreER mice, which express a tamoxifen-inducible Cre recombinase ubiquitously[21]. Double-transgenic mice were injected with tamoxifen (0.5 mg i.p.) on P7, inducing high-frequency recombination of the gene segment encoding CAG-Z-EGFP-caALK2 throughout hindlimb muscle and vascular and connective tissues, as indicated by the loss of nuclear β-galactosidase staining (**Fig. 4c**). Muscle tissues undergoing recombination by this technique did not show edema or mononuclear cell infiltrates. In contrast to Ad.Cre-induced recombination, global postnatal overexpression of $ALK2^{Q207D}$ did not lead to detectable radiological ossification by P60 (**Fig. 4d**). When double-transgenic mice were injected with tamoxifen on P7 and injected with a control adenovirus (Ad.GFP, 1×10^8 PFU, left popliteal fossa) on P8, mildly decreased left hindlimb range of motion and small ectopic calcifications appeared by P14 (**Fig. 4e**). These data suggest that expression of caALK2 is by itself insufficient to produce ectopic bone, and that inflammation or tissue injury from viral immunogenicity or cytotoxicity might be required for bone formation. To test this hypothesis, we

treated conditional caALK2–expressing mice with dexamethasone (10 mg kg^{-1} d^{-1} i.p.) starting 1 d after Ad.Cre injection at P7 and assessed them for ectopic calcification. By P30, ectopic calcifications and immobility were markedly reduced in corticosteroid-treated mice compared with vehicle-treated mice (**Fig. 4f,g**). The impact of corticosteroid treatment suggests that inflammation and caALK2 expression are both required to form ectopic bone. Unlike LDN-193189 administration, dexamethasone administration was accompanied by toxicity, reflected by severely impaired weight gain during drug administration (**Supplementary Figs. 5** and **7** online).

These studies provide a model of heterotropic ossification that recapitulates key features of human FOP, including the evolution of intramuscular endochondral bone and radiological and functional outcomes. Episodes of ectopic ossification in afflicted humans are frequently precipitated by viral prodromes and accompanying viral myositis[22]. Formation of ectopic bone in conditional caALK2–transgenic mice in conjunction with local adenovirus infection and the

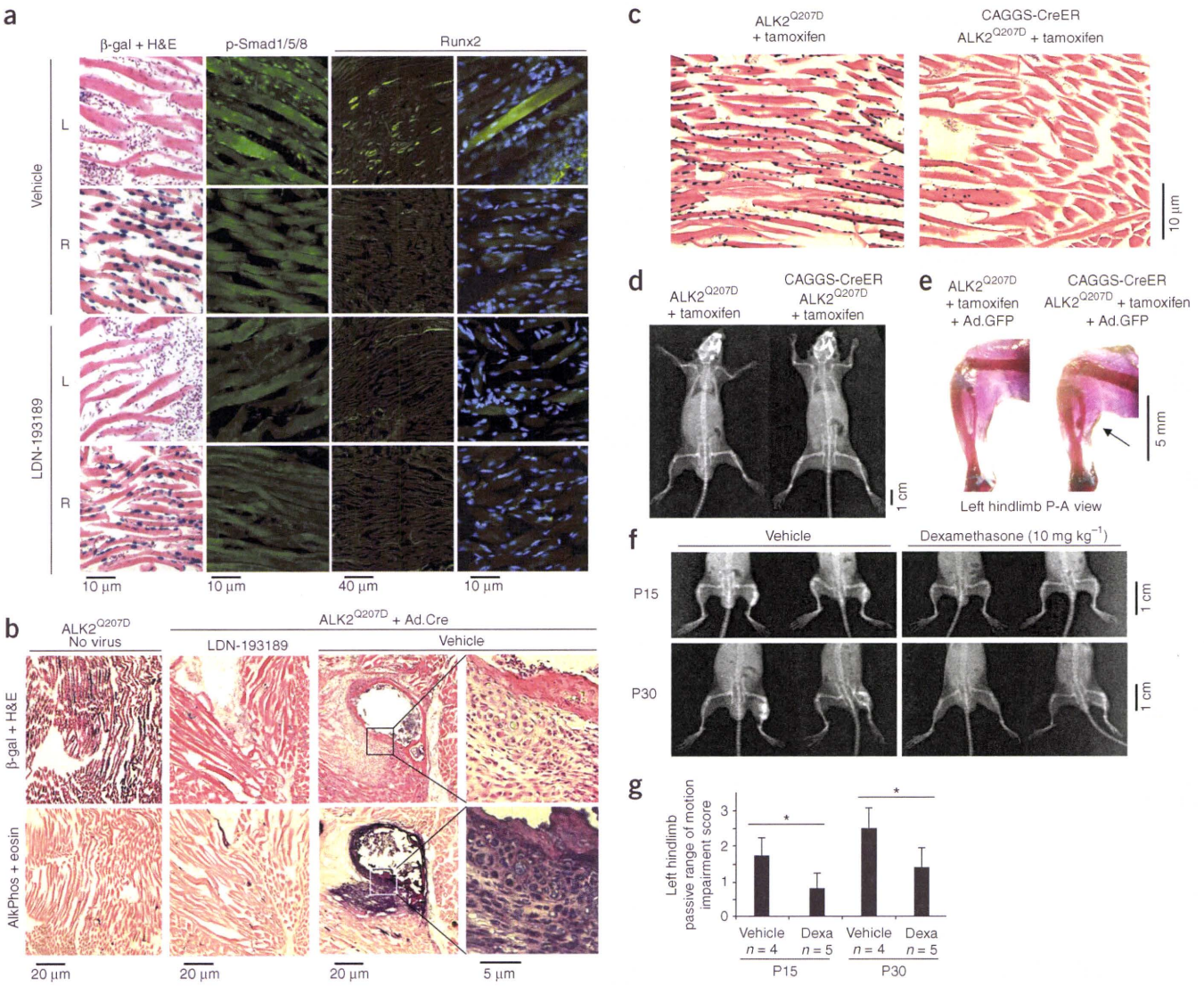

Figure 4 Impact of pharmacologic inhibition of BMP signaling and inflammation in the mouse FOP model. (**a**) Immunofluorescence showing enhanced nuclear p-Smad1/5/8 and expression of Runx2 in a number of recombined (β-gal–negative) myocytes from left (L) hindlimb muscles of Ad.Cre-injected, conditional caALK2–mutant mice at P11 (with DAPI counterstain in blue, right). Both are diminished in LDN-193189–treated mice. Uninjected right(R)-hindlimbs are shown as controls. (**b**) Histological evidence of intramuscular endochondral bone, as shown by alkaline phosphatase staining (AlkPhos) of osteoblasts, chondrocytes, matrix and marrow cells in recombined tissues of vehicle-treated, Ad.Cre-injected, caALK2-transgenic mice at P30. The staining is diminished and absent in LDN-193189–treated and uninfected mice, respectively (higher magnification, right panels). (**c**) High-frequency recombination evidenced by loss of β-gal staining in muscle, vascular and connective tissues of tamoxifen-treated, CAGGS-CreER:CAG-Z-EGFP-caALK2 mice but not single-transgenic CAG-Z-EGFP-caALK2 mice at P30. (**d**) Plain radiographs of tamoxifen-treated double- and single-transgenic mice at P60 show absence of ectopic calcification. (**e**) Alizarin red and Alcian blue staining revealing ectopic calcification in tamoxifen-treated, Ad.GFP-injected double-transgenic but not single-transgenic mice at P14. (**f**) Impact of dexamethasone treatment (10 mg kg^{-1} daily) upon radiographic ossifications in Ad.Cre-injected, conditional caALK2–expressing mice at P15 and P30, as compared to vehicle treatment. (**g**) Impact of dexamethasone treatment upon impairment of passive range of motion (ankle flexion) in Ad.Cre-injected, conditional caALK2–expressing mice at P15 and P30 (data are representative of three independent experiments, *n* as indicated; values are mean ± s.d., $^*P < 0.05$).

response to corticosteroid treatment support a similar relationship between ossification and inflammation in the mouse model as suggested in previous reports[23,24]. These studies confirm the role of dysregulated ALK2 kinase activity in the molecular pathogenesis of FOP[19] while demonstrating the potential of rational therapy mediated through inhibition of ALK2. Despite this promising result, it is worthwhile to note that before any human therapy can be considered using this approach, comprehensive and long-term toxicity studies in multiple species and further drug refinement and optimization will be necessary to ensure adequate safety of both the compound and chronic or intermittent inhibition of BMP signaling *in vivo*. Moreover, the ability of LDN-193189 to prevent ectopic ossification in conditional caALK2–transgenic mice was incomplete. It is possible that LDN-193189, in the manner it was administered, was insufficient to completely suppress activation of SMAD signaling. It is also conceivable that activation of SMAD-independent pathways, which were not blocked by dorsomorphin (ref. 16) and may not be inhibited by LDN-193189, can contribute to the ectopic ossification associated with caALK2 expression.

BMP signals have pivotal roles in vertebrate development, regulating gastrulation, patterning and organogenesis by specifying the fate of multipotent cells[25]. In contrast, the postnatal roles of BMP signals are most essential in the contexts of injury repair, inflammation or remodeling, in the skeleton as well as in connective and vascular tissues[26,27]. Given the apparent requirement for injury and enhanced BMP signaling for FOP lesions in humans and mice, it is possible that abnormal ossification results from diversion of an injury repair program toward bone formation, perhaps deviating the normal adaptive response of cells with regenerative potential. Understanding the cellular basis of endochondral bone formation in FOP could yield insights into the mechanism of heterotopic ossification of autoimmune, post-traumatic or postsurgical etiologies and reveal how BMPs specify plasticity of mesenchyme-derived tissues in physiology and in disease.

METHODS

Chemical, protein and viral agents. We purchased dorsomorphin (Compound C, 6-[4-(2-piperidin-1-yl-ethoxy)phenyl]-3-pyridin-4-yl-pyrazolo[1,5-a]pyrimidine) from EMD Biosciences. We synthesized LDN-193189 (4-[6-(4-piperazin-1-ylphenyl)pyrazolo[1,5-a]pyrimidin-3-yl]quinoline) as previously described[6], determined its purity (99.8%) by HPLC and confirmed its structure by ^{1}H-NMR and high-resolution mass spectrometry. The vehicle was 2% (wt/vol) (2-hydroxypropyl)-β-cyclodextrin in PBS, pH 7.4. We purchased dexamethasone from Sigma. Recombinant human BMP4, platelet-derived growth factor-BB and TGF-β were obtained from R&D Systems. We produced adenoviruses expressing GFP and Cre and quantified them by the plaque-titer method.

Conditionally-expressed, constitutively-active ALK2–transgenic mice. The construction of mice expressing a single conditionally expressed allele of the gene encoding constitutively-active ALK2^{Q207D} (CAG-Z-EGFP-caALK2) on a C57BL/6 background was previously described[14]. We obtained CAGGS-CreER mice, which express a tamoxifen-inducible Cre recombinase ubiquitously under the control of the cytomegalovirus immediate-early enhancer and the chicken β-actin promoter/enhancer[20], from the Jackson Laboratory. All mouse experiments were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care.

Cell culture. We isolated PASMCs from wild-type and CAG-Z-EGFP-caALK2–transgenic mice as previously described[28] and cultured them in RPMI medium (Invitrogen) supplemented with 10% FBS. We induced recombination of PASMCs expressing conditional caALK2 *in vitro* by infecting with Ad.Cre (multiplicity of infection of 50) or Ad.GFP as a control and then culturing for 3 d and passaging. We cultured C2C12 myofibroblast cells (American Type Culture Collection) in DMEM (Invitrogen) supplemented with glutamine and 10% FBS. We preincubated cells with pharmacological inhibitors for 10 min and then exposed them to BMP4, TGF-β or platelet-derived growth factor-BB ligands for 30 minutes at 37 °C.

Immunoblot analysis of Smad1, Smad5 and Smad8 phosphorylation. We mechanically homogenized cell extracts in SDS-lysis buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 50 mM dithiothreitol and 0.01% bromophenol blue), separated the proteins by SDS-PAGE, immunoblotted with polyclonal antibodies specific for phosphorylated Smad1, Smad5 and Smad8, phosphorylated Smad2 (Cell Signaling Technology) or rabbit monoclonal antibodies specific for Smad1 (Epitomics) or Smad2 (Cell Signaling), and visualized the immunoreactive proteins with ECL Plus (GE Healthcare).

Alkaline phosphatase activity. We seeded C2C12 cells into 96-well plates at 2,000 cells per well in DMEM supplemented with 2% FBS. We treated the wells in quadruplicate with BMP ligands and LDN-193189 or vehicle. We collected the cells after 6 d in culture in 50 μl Tris-buffered saline and 1% Triton X-100. We added the lysates to *p*-nitro-phenylphosphate reagent in 96-well plates (Sigma) for 1 h and then evaluated alkaline phosphatase activity (absorbance at 405 nm). We measured cell viability and quantity by Cell Titer Aqueous One (absorbance at 490 nm, Promega), using replicate wells treated identically to those used for alkaline phosphatase measurements.

Visualization of skeletal mineralization. We fixed soft tissues and bone and stained them with Alizarin red and Alcian blue as previously described[29].

Radiography. For plain film radiography, we anesthetized the mice with ketamine and xylazine and then immobilized them and X-rayed them. For three-dimensional images, we killed the mice and analyzed them with a desktop microtomographic imaging system (μCT40, Scanco Medical AG) equipped with a 10-mm focal spot microfocus X-ray tube. We acquired transverse CT slices of the lower pelvis and hindlimbs with a 12-μm isotropic voxel size. We reconstructed, filtered and thresholded the images with a specimen-specific threshold.

Histology. We killed mice and fixed their limbs in 0.5% paraformaldehyde in PBS overnight and then decalcified them in 14% EDTA disodium salt solution with daily changes for 3 d. We incubated the limbs overnight in 30% sucrose with PBS, embedded them in optimal cutting-temperature medium and prepared 12-μm sections with the Cryo-Jane system (Instrumedics). We stained sections for β-galactosidase or alkaline phosphatase activity (X-Gal and BM purple stains, respectively, Roche), and/or with hematoxylin or eosin counterstains. For immunofluorescence, we post-fixed sections in cold methanol to disrupt GFP fluorescence and incubated them with polyclonal antibodies specific for phosphorylated Smad1, Smad5 and Smad8 (1:100, Cell Signaling) or Runx2 (M-70, 1:200, Santa Cruz Biotechnology), followed by Alexa Fluor 488–labeled goat Fab specific for rabbit IgG (Invitrogen).

Id1 and plasminogen activator inhibitor-1 promoter luciferase reporter assays. We transiently transfected mouse PASMCs grown to 50% confluence in six-well plates with 0.3 μg *Id1* promoter luciferase reporter construct (BRE-Luc[30], kindly provided by P. ten Dijke) in combination with 0.6 μg of plasmid expressing constitutively active forms of BMP type I receptors (caALK2, caALK3 or caALK6[31], kindly provided by K. Miyazono), using Fugene6 (Roche). To assess activin and TGF-β type I receptor function, we transiently transfected PASMCs with 0.3 μg *PAI1* (plasminogen activator inhibitor-1) promoter luciferase reporter construct (CAGA-Luc[32], provided by P. ten Dijke) in combination with 0.6 μg of plasmid expressing constitutively active forms of type I receptors (caALK4, caALK5 and caALK7[33], provided by K. Miyazono). For both reporter plasmids, we used 0.2 μg of pRL-TK *Renilla* luciferase (Promega) to control for transfection efficiency. We incubated PASMCs with LDN-193189 (2 nM–32 μM) or vehicle starting 1 h after transfection. We harvested cell extracts and quantified relative promoter activity by the ratio of firefly to *Renilla* luciferase activity with the dual luciferase assay kit (Promega).

Range of motion analyses. To quantify impaired mobility via passive range of motion, we anesthetized transgenic and control mice and assessed them for the ability to passively dorsiflex the left ankle joint. Scores were assessed by two independent observers blinded to genotype and treatment. The observers scored the minimum angle formed by the ankle and the tibia with passive dorsiflexion under light manual pressure as follows: 0, normal flexion with a minimal angle of $< 20°$; 1, mildly impaired flexion with a minimal angle of $\geq 20°$ but $< 90°$; 2, moderately impaired flexion with a minimal angle of $\geq 90°$ but $< 135°$; and 3, severely impaired flexion with a minimal angle of $\geq 135°$ (depicted in **Fig. 3f**).

Statistical analyses. We measured the statistical significance of compared measurements with the Student's two-tailed *t*-test.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

P.B.Y. wrote the manuscript. P.B.Y., D.Y.D. and C.S.L. designed and performed experiments and analyzed data. G.D.C., P.B.Y., K.D.B. and R.T.P. helped to design, synthesize and evaluate dorsomorphin derivatives, and G.D.C. provided pharmacokinetic data. M.L.B. provided technical expertise and μCT tomography data. D.W.H. and P.M.M. performed experiments. C.S. tested the efficacy of the dorsomorphin derivative with additional assays. N.K. performed control experiments. Y.M. and T.F. provided key experimental reagents. T.K. provided reagents and experimental advice. C.C.H., Y.M. and K.D.B. provided feedback and experimental advice, and P.B.Y. and K.D.B. edited the manuscript.

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1. Shore, E.M. & Kaplan, F.S. Insights from a rare genetic disorder of extra-skeletal bone formation, fibrodysplasia ossificans progressiva (FOP). *Bone* **43**, 427–433 (2008).
2. Buyse, G., Silberstein, J., Goemans, N. & Casaer, P. Fibrodysplasia ossificans progressiva: still turning into wood after 300 years? *Eur. J. Pediatr.* **154**, 694–699 (1995).
3. Kaplan, F.S., Glaser, D.L., Pignolo, R.J. & Shore, E.M. A new era for fibrodysplasia ossificans progressiva: a druggable target for the second skeleton. *Expert Opin. Biol. Ther.* **7**, 705–712 (2007).
4. Kaplan, F.S. *et al.* Fibrodysplasia ossificans progressiva. *Best Pract. Res. Clin. Rheumatol.* **22**, 191–205 (2008).
5. Shore, E.M. *et al.* A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nat. Genet.* **38**, 525–527 (2006).
6. Cuny, G.D. *et al.* Structure-activity relationship study of bone morphogenetic protein (BMP) signaling inhibitors. *Bioorg. Med. Chem. Lett.* **18**, 4388–4392 (2008).
7. Tsuchida, K., Mathews, L.S. & Vale, W.W. Cloning and characterization of a transmembrane serine kinase that acts as an activin type I receptor. *Proc. Natl. Acad. Sci. USA* **90**, 11242–11246 (1993).
8. Miyazono, K., Maeda, S. & Imamura, T. BMP receptor signaling: Transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine Growth Factor Rev.* **16**, 251–263 (2005).
9. Groppe, J.C., Shore, E.M. & Kaplan, F.S. Functional modeling of the ACVR1 (R206H) mutation in FOP. *Clin. Orthop. Relat. Res.* **462**, 87–92 (2007).
10. Shore, E.M., Xu, M., Connor, J.M. & Kaplan, F.S. Mutations in the BMP type I receptor ACVR1 in patients with fibrodysplasia ossificans progressiva (FOP). *J. Bone Miner. Res.* **21**, S75 (2006).
11. Macias-Silva, M., Hoodless, P.A., Tang, S.J., Buchwald, M. & Wrana, J.L. Specific activation of Smad1 signaling pathways by the BMP7 type I receptor, ALK2. *J. Biol. Chem.* **273**, 25628–25636 (1998).
12. Zhang, D. *et al.* ALK2 functions as a BMP type I receptor and induces Indian hedgehog in chondrocytes during skeletal development. *J. Bone Miner. Res.* **18**, 1593–1604 (2003).
13. Koefoed, M. *et al.* Biological effects of rAAV-caAlk2 coating on structural allograft healing. *Mol. Ther.* **12**, 212–218 (2005).
14. Fukuda, T. *et al.* Generation of a mouse with conditionally activated signaling through the BMP receptor, ALK2. *Genesis* **44**, 159–167 (2006).
15. Waheed, I. *et al.* Factors associated with induced chronic inflammation in mdx skeletal muscle cause posttranslational stabilization and augmentation of extrasynaptic sarcolemmal utrophin. *Hum. Gene Ther.* **16**, 489–501 (2005).
16. Yu, P.B. *et al.* Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat. Chem. Biol.* **4**, 33–41 (2008).
17. Yu, P.B. *et al.* Bone morphogenetic protein (BMP) type II receptor is required for BMP-mediated growth arrest and differentiation in pulmonary artery smooth muscle cells. *J. Biol. Chem.* **283**, 3877–3888 (2008).
18. Billings, P.C. *et al.* Dysregulated BMP signaling and enhanced osteogenic differentiation of connective tissue progenitor cells from patients with fibrodysplasia ossificans progressiva (FOP). *J. Bone Miner. Res.* **23**, 305–313 (2008).
19. Fukuda, T. *et al.* Constitutively activated ALK2 and increased smad1/5 cooperatively induce BMP signaling in fibrodysplasia ossificans progressiva. *J. Biol. Chem.* published online, doi:10.1074/jbc.M801681200 (6 August 2008).
20. Hegyi, L. *et al.* Stromal cells of fibrodysplasia ossificans progressiva lesions express smooth muscle lineage markers and the osteogenic transcription factor Runx2/Cbfa-1: clues to a vascular origin of heterotopic ossification? *J. Pathol.* **201**, 141–148 (2003).
21. Hayashi, S. & McMahon, A.P. Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. *Dev. Biol.* **244**, 305–318 (2002).
22. Scarlett, R.F. *et al.* Influenza-like viral illnesses and flare-ups of fibrodysplasia ossificans progressiva. *Clin. Orthop. Relat. Res.* 275–279 (2004).
23. Kaplan, F.S. *et al.* Hematopoietic stem-cell contribution to ectopic skeletogenesis. *J. Bone Joint Surg. Am.* **89**, 347–357 (2007).
24. Pignolo, R.J., Suda, R.K. & Kaplan, F.S. The fibrodysplasia ossificans progressiva lesion. *Clin. Rev. Bone Miner. Metab.* **5**, 195–200 (2005).
25. Zhao, G.Q. Consequences of knocking out BMP signaling in the mouse. *Genesis* **35**, 43–56 (2003).
26. Tsuji, K. *et al.* BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. *Nat. Genet.* **38**, 1424–1429 (2006).
27. Corriere, M.A. *et al.* Endothelial Bmp4 is induced during arterial remodeling: effects on smooth muscle cell migration and proliferation. *J. Surg. Res.* **145**, 142–149 (2008).
28. Yu, P.B., Beppu, H., Kawai, N., Li, E. & Bloch, K.D. Bone morphogenetic protein (BMP) type II receptor deletion reveals BMP ligand-specific gain of signaling in pulmonary artery smooth muscle cells. *J. Biol. Chem.* **280**, 24443–24450 (2005).
29. Komori, T. *et al.* Targeted disruption of *Cbfa1* results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* **89**, 755–764 (1997).
30. Korchynskyi, O. & ten Dijke, P. Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the *Id1* promoter. *J. Biol. Chem.* **277**, 4883–4891 (2002).
31. Fujii, M. *et al.* Roles of bone morphogenetic protein type I receptors and Smad proteins in osteoblast and chondroblast differentiation. *Mol. Biol. Cell* **10**, 3801–3813 (1999).
32. Dennler, S. *et al.* Direct binding of Smad3 and Smad4 to critical TGF β-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J.* **17**, 3091–3100 (1998).
33. Shimizu, A. *et al.* Identification of receptors and Smad proteins involved in activin signalling in a human epidermal keratinocyte cell line. *Genes Cells* **3**, 125–134 (1998).

Erratum: BMP type I receptor inhibition reduces heterotopic ossification

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In the version of this article initially published, the title included a misspelling—'heterotropic' should have been 'heterotopic'. Additionally, the fourth and fifth sentences of the abstract were incorrectly worded and have been corrected to state more clearly the role of Ad.Cre. These changes do not affect the scientific content of the text. The errors have been corrected in the HTML and PDF versions of the article.