

Animal Models of Fibrodysplasia Ossificans Progressiva

Frederick S. Kaplan, MD,^{1,2,4} Eileen M. Shore, PhD,^{1,3,4} Robert J. Pignolo, MD, PhD,^{2,4}
and David L. Glaser, MD^{1,4}

Departments of ¹Orthopaedic Surgery, ²Medicine, and ³Genetics, and ⁴The Center for Research in FOP
and Related Disorders, The University of Pennsylvania School of Medicine, Philadelphia, PA

Abstract

Animal models of fibrodysplasia ossificans progressiva (FOP) are important for understanding the pathophysiology of FOP and for testing possible therapies. Laboratory-generated genetic animal models, each with features of FOP, provide the opportunity to better understand the biology of FOP, and to study the effectiveness and safety of currently available and emerging therapies.

Key Words: Fibrodysplasia ossificans progressiva (FOP); BMP4; heterotopic ossification; animal models.

The Importance of Animal Models for Fibrodysplasia Ossificans Progressiva

The emergence of relevant animal models for fibrodysplasia ossificans progressiva (FOP) is critical for the development of definitive treatments (1,2) (Table 1). A sporadic FOP-like condition was described in domestic housecats, but no known living animals have been available for further study (3). The first animal model that provided a clue to the cause of FOP was recognized in the *decapentaplegic* (*dpp*) mutants of *Drosophila*, an invertebrate, and predicted the role of bone morphogenetic protein (BMP)-signaling in the pathophysiology of FOP (4). Studies in c-Fos embryonic stem cell chimeric mice demonstrated progressive heterotopic ossification

(HO) in FOP-like patterns, and suggested an interaction between proto-oncogenes and the BMP-signaling pathway in the molecular pathophysiology of FOP-like lesions (5,6). The homozygous knockout of the genes encoding Noggin (a secreted BMP antagonist) and BMP receptor IB (BMPRII; a type I BMP receptor) in mice elucidated the importance of the BMP signaling pathway in joint morphogenesis, a finding relevant to the joint malformations in FOP (7–9). The BMP4–Matrigel implant model provided a reproducible experimental system for studying the pathology and prevention of FOP-like lesion formation (10). Transgenic mice that overexpress BMP4 under the control of the neuron-specific enolase (Nse) promoter develop progressive HO, but lack the anatomic specificity seen in the human disease (11). The achievement of a genuine animal model for FOP may have to await the discovery of the gene responsible for FOP.

Despite their limitations, the two most useful animal models for the induction of FOP-like lesions are the recombinant BMP4–Matrigel implant system and the Nse–BMP4 transgenic mouse (10,11). Each

Address correspondence to Frederick S. Kaplan, MD, Department of Orthopaedic Surgery, The University of Pennsylvania School of Medicine, Silverstein Two, 3400 Spruce St., Philadelphia, PA 19104. E-mail: frederick.kaplan@uphs.upenn.edu.

Table 1
Comparative Clinical and Pathogenetic Features of Fibrodysplasia Ossificans Progressiva
and Several Relevant Animal Models

Clinical or pathogenetic feature	FOP	FOP-like features in domestic house cat	c-Fos embryonic stem cell chimera	Noggin knockout	BMP4–Matrigel implant model	Nse–BMP4 transgenic animal model
Naturally occurring	Yes	Yes	No	No	No	No
Transgenic animal model	No	No	No	No	No	Yes
Genetic knockout	No	No	No	Yes	No	No
Stem cell chimera	No	No	Yes	No	No	No
Pathophysiology of heterotopic ossification	Unknown	Unknown	Increased embryonic expression of Fos	Homozygous deletion of <i>Noggin</i> gene	BMP4-induced	Neuron-specific overexpression of BMP4
General appearance at birth	Healthy	Healthy	Healthy	Lethal Yes; other digital and joint anomalies	Healthy	Healthy
Great toe malformation	Yes	No	No		No	No
Congenital heterotopic ossification	No	Yes	No	Yes	No	No
Postnatal heterotopic ossification	Yes	Yes	Yes	Unknown	Yes	Yes
Progressive heterotopic ossification	Yes	Yes	Yes	Unknown	No	Yes
Progression according to anatomic patterns seen in FOP	Yes	Yes	Yes	Unknown	No	Generally opposite
Progressive chest wall disease	Yes	Yes	Yes	Present at birth	No	Yes

(table continues)

of these in vivo model systems reproduce all of the histological stages of FOP lesion formation (1,12). Each of these models has been used to study the early histological events associated with BMP-induced HO and to test the effects of BMP antagonists, such as Noggin, in inhibiting the formation of FOP-like lesions (1,12). The BMP4–Matrigel implant model and the Nse–BMP4 transgenic mouse are useful in vivo model systems to assess currently

available treatments for FOP and to help understand the pathophysiology of progressive HO until a better animal model can be developed based on the precise gene mutation causing FOP.

FOP: A Clue From the Fly

BMPs were first described by Marshall Urist in 1965 as a fraction of bone matrix capable of inducing

Table 1
(continued)

Clinical or pathogenetic feature	FOP	FOP-like features in domestic house cat	c-Fos embryonic stem cell chimera	Noggin knockout	BMP4–Matrigel implant model	Nse–BMP4 transgenic animal model
Progressive restriction of joint movement	Yes	Yes	Yes	Joints do not form	No	Yes
Exacerbation by trauma	Yes	Unknown	No	Unknown	No	Unknown
Muscles spared from disease process	Diaphragm, extra-ocular cardiac and smooth	Same as FOP	Same as FOP	Same as FOP	Not applicable	Same as FOP
Orthotopic fusions of cervical spine	Yes	No	No	Yes	No	No
Proximal medial tibial osteochondromas	Yes	No	No	Yes	No	No
Hearing impairment/conductive hearing loss	Yes	Unknown	Unknown	Unknown	No	Unknown
Lymphocyte infiltrate in skeletal muscles	Yes	Yes	Yes	Yes	Yes	Yes
Fibroproliferative angiogenic lesions	Yes	Yes	Yes	Yes	Yes	Yes
Endochondral ossification	Yes	Yes	Yes	Yes	Yes	Yes
BMP pathway abnormalities	Yes	Yes	Yes	Yes	Yes	Yes

ossification following implantation at a heterotopic site (13). After the murine and human BMP genes were cloned and recombinant proteins expressed, it became clear that recombinant BMPs could induce the entire cascade of endochondral bone formation at a heterotopic site (14). Furthermore, the protein-coding regions of BMP2 and BMP4 were strikingly similar to the protein-coding region of the dipteran gene *dpp* (4). Pleiotropic mutations of the *dpp* locus led to disturbances in the body plan of *Drosophila* that were similar in scope and pattern to those seen in patients with FOP (4). A remarkable conservation of a pro-

tein sequence across so large an evolutionary distance (that between humans and flies) suggested that the *BMP2* and *BMP4* genes in humans and the *dpp* gene in *Drosophila* are likely derived from a common ancestral gene. The developmental similarities between the mutant *dpp* phenotypes in the fly and FOP in humans suggested a useful model for understanding FOP. The model was especially fruitful in suggesting a molecular basis for FOP, and provided the insight that linked bone formation with pattern abnormalities before vertebrate animal models were available to study the BMP signaling pathway (4).

A FOP-Like Condition in the Cat

A FOP-like condition has been described in domestic housecats, and six sporadic cases have been reported (3). The disease occurs in male and female cats, and is seen in both the domestic short-hair and domestic long-hair cats. Affected cats ranged from 10 mo to 6 yr of age. Unlike FOP in humans, affected cats do not have congenital malformations of the distal limbs. Radiographic examination revealed multiple areas of HO within affected muscles. Intense perivascular lymphocytic infiltration was noted at the advancing edge of fibroproliferative lesions and was similar to that seen in FOP lesions in humans. In the feline lesions, there was a marked proliferation of connective tissue, followed by cartilage and bone formation within epimysium, tendons, and fasciae similar to the early appearance of FOP in man. The clinical course of the feline disease was rapid, with the development of severe disability occurring within weeks to months. The FOP-like disease in the cat closely mimics FOP in humans and may serve as a natural animal model for this disorder (3,12). Unfortunately, all evaluations performed to date have been postmortem studies on pet cats, and no live animals are currently available for study.

A FOP-Like Condition in c-Fos Stem Cell Chimeric Mice

Murine embryonic overexpression of the c-Fos proto-oncogene leads to early progressive, postnatal heterotopic chondrogenesis and osteogenesis with phenotypic features similar to those seen in children who have FOP (5,6). The overexpression of c-Fos in embryonic stem cell chimeras leads to progressive heterotopic endochondral osteogenesis at least, in part, through a BMP4-signaling pathway. In contrast, early FOP lesions express abundant BMP4 without abundant expression of c-Fos messenger RNA, suggesting that the primary molecular defect in FOP may be independent of the sustained c-Fos effects on chondrogenesis and osteogenesis. Comparisons of the clinical, molecular, and pathogenetic features of the c-Fos embryonic stem cell chimeras with those of FOP provide insight into the earliest events in the molecular pathogenesis of genetically induced heterotopic chondrogenesis and

osteogenesis. The relevance of the c-Fos embryonic stem cell chimera to the study of FOP demonstrates the power of using embryonic stem cell technology to generate gain-of-function mutations in the study of FOP. It also illustrates the potential interaction between proto-oncogenes and BMPs in the pathophysiology of progressive HO (5,6).

FOP-Like Features in Noggin Knockout Mice and in BMPRIB Knockout Mice

BMP4, a potent skeletal morphogen, is overexpressed in the cells of patients who have FOP (15,16). Additionally, Noggin, a secreted protein that functions as a high-affinity antagonist of BMP4 and several other BMPs, is underexpressed in the cells of patients who have FOP (17). Noggin acts by binding BMPs and preventing the interaction of BMPs with their serine-threonine kinase transmembrane receptors (18). During vertebrate development, Noggin expression and secretion is triggered by BMP signaling, and acts to define the boundaries of BMP-induced structures. As part of a negative feedback control system, BMPs regulate the expression of Noggin (17). Homozygous deletion of the *Noggin* gene in mice is lethal soon after birth owing to multiple congenital skeletal defects. The skeletons of *Noggin* knockout mice have multiple synostoses, ankylosis of the chest wall, and fail to form joints throughout the body. In mice lacking Noggin, cartilage condensations formed normally, but initiation of joint development failed to occur (9).

Interestingly, homozygous deletions of the *BMPRIB* gene leads to essentially normal mice, except for the presence of digital malformations similar to those seen in patients with FOP and brachydactyly type A2 (7,8). These findings from the *Noggin* knockout mice and the *BMPRIB* knockout mice demonstrate the importance of BMP signaling, not only in HO, but also in joint morphogenesis.

BMP4-Matrigel Implant Model

The BMP4-Matrigel implant model reproduces all of the stages of HO observed during lesion formation in patients who have FOP (10). Although the BMP4-Matrigel implant animal model lacks the

specificity of progressive HO, it permits the study of the various histological stages of lesion development, the study of tissue-specific target cells responsible for FOP-like lesions, and provides a test platform for drug development (10).

A FOP-Like Phenotype in Nse-BMP4 Transgenic Mice

Transgenic mice overexpressing BMP4 under the control of the Nse promoter develop a FOP-like phenotype and provide an important new model system for studying the pathophysiology of progressive HO (11).

Heterotopic bone formation in the transgenic animals follows the same endochondral ossification pathway seen in the BMP4-Matrigel implant model and in patients who have FOP. HO in Nse-BMP4 transgenic mice begins subcutaneously in the connective soft-tissue structures, rather than in deep skeletal muscle, and is similar to the early FOP involvement of the fascia, tendons, and aponeuroses. Heterotopic bone formation in the transgenic animals is progressive. However, the patterns of disease progression are different from those seen in FOP. Interestingly, the Nse-BMP4 transgenic mice fail to form heterotopic bone in the diaphragm, tongue, or extraocular muscles—muscles that are also spared in FOP. Nse-BMP4 transgenic mice lack the great toe and other joint malformations seen in patients with FOP patients. The lack of joint malformations in the Nse-BMP4 transgenic mice might be the result of the late embryonic transgenic expression of BMP4 at a time following the establishment of the cartilaginous anlage.

When the Nse-BMP4 transgenic mice were mated with Nse-Noggin transgenic animals, the progressive HO was prevented, thus confirming the role of the BMP4 signaling pathway in the pathogenesis of the progressive HO in these animals. The Nse-BMP4 transgenic animal model provides important insight into the pathogenesis of FOP-like lesions and provides an *in vivo* model for testing medications that might slow the progression of HO (11).

Summary

Animal models have illuminated the role of the BMP4 signaling pathway in pattern formation and

in HO, and have provided *in vivo* model systems for testing drugs that may inhibit BMP4-induced HO. Each animal model has its potentials and limitations for understanding the developmental biology of FOP and for studying the evolution of postnatal HO. The eventual identification of the FOP gene will hasten the development of a true animal model of FOP.

References

1. 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 Ed. Wiley-Liss; John Wiley & Sons, New York, pp. 827–840.
2. Kaplan FS, Glaser DL, Hebel N, Shore EM. 2004 Heterotopic ossification. *J Am Acad Orthop Surg* 12:116–125.
3. Valentine BA, Kaplan FS. 1996 Fibrodysplasia ossificans progressiva in cats: a potentially important animal model of the human disease. *Feline Practice* 24:6.
4. Kaplan FS, Tabas JA, Zasloff MA. 1990 Fibrodysplasia ossificans progressiva: A clue from the fly? *Calcif Tiss Int* 47:117–125.
5. Kaplan FS, Shore EM. 1996 Bone morphogenetic protein and c-fos: early signals in endochondral bone formation. *Bone* 19:S513–S521.
6. Olmsted EA, Gannon FH, Wang Z-Q, et al. 1998. Embryonic overexpression of the c-fos proto-oncogene: a murine stem cell chimera applicable to the study of fibrodysplasia ossificans progressiva in humans. *Clin Orthop Rel Res* 346:81–94.
7. Lehmann K, Seemann P, Stricker S, et al. 2003 Mutations in bone morphogenetic protein receptor IB cause brachydactyly type A2. *Proc Natl Acad Sci USA* 100:12,277–12,282.
8. Yi SE, Daluiski A, Pederson R, Rosen V, Lyons KM. 2000 The type I BMP receptor BMPRII is required for chondrogenesis in the mouse limb. *Development* 127: 621–630.
9. Brunet LJ, McMahon JA, McMahon AP, Harland RM. 1998 Noggin, cartilage morphogenesis, and joint formation in the mammalian skeleton. *Science* 280:1455–1457.
10. Glaser DL, Economides AN, Wang L, et al. 2003 *In vivo* somatic cell gene transfer of an engineered noggin mutein prevents BMP4-induced heterotopic ossification. *J Bone Joint Surg* 85-A:2332–2342.
11. 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.
12. 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 Rel Res* 346:19–25.
13. Urist MR. 1965 Bone formation by autoinduction. *Science* 150:893–899.

14. Wozney JM, Rosen V, Celeste AJ, et al. 1988 Novel regulators of bone formation of bone formation: molecular clones and activities. *Science* 242:1528–1534.
15. Shafritz AB, Shore EM, Gannon FH, et al. 1996 Overexpression of an osteogenic morphogen in fibrodysplasia ossificans progressiva. *N Engl J Med* 335: 555– 561.
16. 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.
17. 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* 85-A:667–674.
18. Groppe J, Greenwald J, Wiater E, et al. 2002 Structural basis of BMP signaling inhibition by the cystine knot protein Noggin. *Nature* 420:636–642.