

The Neuron-Specific Enolase–Bone Morphogenic Protein 4 Transgenic Mouse

A Fibrodysplasia Ossificans Progressiva-Like Animal Model

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Abstract

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder characterized by congenital malformation of the great toes and by progressive, postnatal heterotopic bone formation. Although the genetic defect in FOP is not known, several lines of evidence suggest that dysregulation of bone morphogenetic protein (BMP) 4 may be involved in the pathophysiology of the condition. Transgenic mice that overexpress BMP4 under the control of the neuron-specific enolase (Nse) promoter is the first mouse model to develop progressive, postnatal heterotopic endochondral ossification. The Nse–BMP4 transgenic mouse provides a unique opportunity to study the pathophysiology and treatment of progressive heterotopic ossification in an animal model relevant to the study of FOP.

Key Words: Bone morphogenic protein 4; transgenic mice; fibrodysplasia ossificans progressiva (FOP); animal model; neuron-specific enolase; heterotopic ossification.

Introduction

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder of congenital toe malformations and progressive heterotopic ossification. The earliest pathological finding in FOP is perivascular lymphocytic infiltration into normal-appearing skeletal muscle, followed by muscle cell degeneration and the emergence of highly vascular fibroproliferative tissue with associated soft-tissue swelling. The fibroproliferative lesions evolve through an endochondral ossification process into mature lamellar bone with marrow elements. Heterotopic ossification (HO) is often first detected around the spine and proximal limbs, then at multiple other sites, which leads to joint dysfunction and near-complete immobility (1).

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Although the genetic cause of FOP has not been identified, several studies have suggested that bone morphogenetic protein (BMP)4 may play a key pathophysiological role in this disease (1–3). Nevertheless, transgenic mice overexpressing BMP4 under the control of different promoters have previously failed to develop a disorder of extraskeletal bone formation (4–7). Recently, developmentally associated, postnatal HO was reported in a transgenic mouse model that overexpresses BMP4 under the control of the neuron-specific enolase (Nse) promoter. These Nse–BMP4 transgenic mice develop progressive postnatal HO through an endochondral process (8).

Features of the Transgenic Model

Transgenic animals that overexpress BMP4 under the control of the Nse promoter were generated to examine the role of BMP signaling in brain development. The overexpression of BMP4 resulted in an

Table 1
Anatomic Patterns of Progressive Heterotopic Ossification
in Patients With FOP and in the Nse-BMP4
Transgenic Mice

FOP	Nse-BMP4 transgenic mice
Axial → Appendicular	Appendicular → Axial
Dorsal → Ventral	Ventral → Dorsal
Cranial → Caudal	Caudal → Cranial
Upper limbs → Lower limbs	Lower Limbs → Upper limbs
Proximal → Distal	Proximal → Distal

increase in the density of astrocytes in multiple brain regions accompanied by a decrease in the density of oligodendrocytes (9). Interestingly, the BMP4 transgenic mice also developed severe postnatal heterotopic bone formation.

The first evidence of a musculoskeletal phenotype in the Nse-BMP4 transgenic mice was swelling of proximal hind limb muscles with enlargement of the hind limb circumference. Histological and immunohistochemical analysis of tissue sections from hind limbs found that the earliest histological changes were the infiltration of CD45+ mononuclear cells into intramuscular regions accompanied by muscle fiber degeneration. Subsequent findings included the proliferation of fibroblast-like cells that matured into HO through a classic endochondral ossification pathway (8).

Heterotopic bone formation in Nse-BMP4 transgenic animals was progressive and followed specific anatomic patterns of progression. HO was primarily located in the subcutaneous connective tissue in a pattern nearly opposite to that seen in patients with FOP (Table 1). Osseous bridges developed in multiple locations, such as the pelvis and the jaw, and eventually immobilized the mice. Kyphoscoliosis and spinal deformity developed frequently. The diaphragm, tongue, and extraocular muscles were spared in the transgenic animals, which also occurs in patients who have FOP. Mating of Nse-BMP4 transgenic mice with transgenic animals that overexpressed the BMP4 antagonist, Noggin (Nse-Noggin transgenics), prevented the disorder, confirming the specific role of BMP4 in the pathogenesis of the disease in this animal model.

Although the Nse-BMP4 animal model closely recapitulates major aspects of the FOP phenotype, there are still substantial phenotypic differences

between this mouse model and human FOP. First, there are no obvious congenital malformations of the great toes found in the transgenic mice. Second, the initial sites of HO and the anatomic patterns of disease progression are different from human FOP (Table 1). Nevertheless, the Nse-BMP4 transgenic animal exhibits progressive heterotopic endochondral ossification, and is relevant to the study of FOP.

Summary

Nse-BMP4 transgenic mice display progressive, postnatal HO and provide a model for future research into the molecular mechanisms and treatment of progressive HO. The Nse-BMP4 animal model supports the hypothesis that dysregulation of BMP4 signaling may play a key pathophysiological role in FOP. Furthermore, the model suggests that a single gene mutation might possibly cause such a variable and multifaceted disorder. Despite the differences in the pattern of affected body locations during disease progression between the mouse and patients who have FOP, this transgenic mouse line is the first transgenic mouse model to exhibit extensive HO. Practically, the Nse-BMP4 animal model can be used as a primary test platform to accelerate drug development for FOP and other disorders of HO.

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. 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.
3. 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-A(4):667–674.
4. Guha U, Gomes WA, Kobayashi T, Pestell RG, Kessler JA. 2002 In vivo evidence that BMP signaling is necessary for apoptosis in the mouse limb. *Dev Biol* 249: 108–120.
5. Blessing M, Nanney LB, King LE, Jones CM, Hogan BL. 1993 Transgenic mice as a model to study the role of TGF-beta related molecules in hair follicles. *Genes Dev* 7:204–215.
6. Bellusci S, Henderson R, Winnier G, Oikawa T, Hogan BL. 1996 Evidence from normal expression and targeted

- misexpression that bone morphogenetic protein (BMP4) plays a role in mouse embryonic lung morphogenesis. *Development* 122:1693–1702.
7. Tsumaki N, Nakase T, Miyaji T, et al. 2002 Bone morphogenetic protein signals are required for cartilage formation and differently regulate joint development during skeletogenesis. *J Bone Miner Res* 17:898–906.
 8. 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.
 9. Gomes WA, Mehler MF, Kessler JA. 2003 Transgenic overexpression of BMP4 increases astroglial and decreases oligodendroglial lineage commitment. *Dev Biol* 255:164–177.

