

## Review Article

# Animal Models of Typical Heterotopic Ossification

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Received 26 August 2010; Accepted 28 September 2010

Academic Editor: Monica Fedele

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Heterotopic ossification (HO) is the formation of marrow-containing bone outside of the normal skeleton. Acquired HO following traumatic events is a common and costly clinical complication. In contrast, hereditary HO is rarer, progressive, and life-threatening. Substantial effort has been directed towards understanding the mechanisms underlying HO and finding efficient treatments. However, one crucial limiting factor has been the lack of relevant animal models. This article reviews the major currently available animal models, summarizes some of the insights gained from these studies, and discusses the potential future challenges and directions in HO research.

## 1. Introduction

Heterotopic ossification (HO) is the formation of marrow-containing bone outside of the normal skeleton [1, 2]. Acquired HO following traumatic events, such as total joint replacements (TJR) [3–5], spinal cord injury (SCI) [6], traumatic brain injury (TBI) [7], fracture, muscular trauma, or war-wounded patients [8, 9], is a common and costly clinical complication. Hereditary HO, such as fibrodysplasia ossificans progressiva (FOP), is rare, progressive, and life threatening [10]. The first description of hereditary HO in FOP was made in 1692 by Guy Patin. Acquired HO as a complication of gunshot wounds was described by Dejerine and Ceillier in 1918 [11]. 16%–53% of SCI (11,000 annually) and TBI (1.4 million) patients and 40%–50% of TJR (1 million) patients will develop HO at some point.

About 10% of HO is symptomatic resulting in limitations in range of motion. Once acquired HO develops, surgical removal is the only effective treatment, normally followed by local radiation or nonsteroidal anti-inflammatory agents (NSAIDs) to prevent recurrence [12]. However, surgical removal is costly, the effectiveness of NSAIDs is variable, and radiation has been associated with malignancies [13, 14]. Further, there is no effective treatment for debilitating hereditary HO, FOP [15].

Substantial effort has been directed towards understanding the mechanisms underlying HO and finding efficient

treatments. However, one crucial limiting factor has been the lack of relevant animal models. This paper reviews the long and arduous efforts to generate clinical relevant animal models and focuses on the features of major currently available models. It also summarizes some of the insights gained from these studies and discusses the potential future challenges and directions in HO research.

For the purposes of this paper, HO is defined as a heterogeneous disorder characterized by pathologic endochondral ossification with hematopoietic bone marrow in soft tissues, such as subcutaneous tissue, skeletal muscle, or fibrous tissue adjacent to joints. Similar pathologies lacking endochondral ossification, such as Progressive Osseous Heteroplasia [15, 16] or that containing no hematopoietic bone marrow, such as ectopic calcification/mineralization [17] (also called dystrophic calcification), are not included.

## 2. Animal Models of Hereditary HO

A typical example of hereditary HO is FOP, which is characterized by stereotyped patterned progressive ossification in soft tissues [18]. In this disorder, mutations in a bone morphogenetic protein (BMP) receptor gene, ACVR1 [19], result in dysregulation of BMP signaling that ultimately leads to FOP [10, 20]. This mutant ACVR1 activates BMP signaling in the absence of BMP ligand leading to BMP-independent chondrogenesis that is enhanced by BMP

ligands [21]. This indicates that mechanism-based animal models that faithfully replicate the disorder virtually require genetic modifications leading to enhanced BMP signaling. Theoretically, there are at least five ways to modify and enhance BMP signaling: (1) by introducing a hyperactive BMP receptor, (2) by knocking out BMP inhibitors, (3) by introducing high level of BMPs, (4) by overexpressing specific BMP target genes, and (5) by modifying BMP signaling indirectly through other factors that can interact with components of BMP signaling pathway.

### 2.1. Animal Models That Introduce Hyperactive BMP Receptor.

Animal models that introduce hyperactive BMP receptor, especially the recently found mutations in ACVR1, would seem to be the most relevant model of FOP. However, introducing a constitutively active ACVR1 mutation into zebrafish embryos failed to induce obvious HO even though strong ventralization due to enhanced BMP signaling was observed [20]. A genetically modified mouse model that carries the same mutation has not yet been reported but will likely be a valuable addition to the field in the future. Interestingly, a number of animals including domestic cats [22–25], shepherd dog [26], pigs [27], and the Southeast Asian mouse deer of the genus *Tragulus* [28] develop FOP-like conditions spontaneously. Even though the exact genetic bases are still unknown, it is reasonable to think that sporadic, spontaneous mutations in the BMP signaling pathway, especially mutations in ACVR1, were likely responsible for the observed FOP-like conditions. Further genetic studies of these affected animals hopefully will clarify this issue. However, due to the rarity of the events and ethical considerations, the practicality of these large animal models as a drug testing platform is in question.

Kobayashi et al. [51] reported that Col2-caBmpr1a transgenic mice that express constitutive active Bmpr1a (caBmpr1a) under the control of rat type II collagen promoter created enhanced BMP signaling. E17.5 transgenic embryos showed severe skeletal abnormalities; the femur, tibia, and patella were fused together, eliminating joint tissues [51]. This study demonstrated that overactive BMP signaling through caBMPR1A in chondrocytes stimulates chondrocyte maturation toward hypertrophic differentiation, but an HO phenotype was not reported in this model. Fukuda et al. reported using a Cre-loxP system to conditionally express a constitutively active ALK2 receptor (caALK2) to activate BMP signaling, but this produced embryonic lethality [29]. Their data indicate that low levels of caAlk2 expression are sufficient to transduce a sufficient amount of BMP signaling to compromise normal development of embryos. We speculate that conditionally activating the caAlk2 expression with late tissue-specific Cre in future studies might generate a mouse model that is useful for study of HO.

**2.2. Animal Models That Knock out BMP Inhibitors.** Genetic deletion of a BMP inhibitor is another strategy for enhancing BMP signaling and potentially producing an animal model with an FOP-like phenotype. In fact, *Noggin*<sup>-/-</sup> mice have some congenital skeletal defects, including congenital HO,

but *Noggin*<sup>-/-</sup> mice die soon after birth [30]. Mice with targeted disruption of *Chordin*, another BMP inhibitor, also die at birth, and they develop defects in inner and outer ear development and show abnormalities in pharyngeal and cardiovascular organization [31]. Mutation of another mouse competitive BMP inhibitor gene, *Gremlin*, resulted also in a severe abnormal skeletal pattern [52]. Interestingly, conditional deletion of *Gremlin* by crossing the floxed mice with osteocalcin promoter-driven cre (*Oc-Cre*) caused only a transient increase in bone formation and bone mass, but not HO [53]. Null mutation of less specific inhibitors of BMP signaling, such as *Dan* [54] or *Cerberus*-like [55], did not generate gross defects; these two mutant lines are born alive and fertile without a postnatal HO phenotype. Thus, although null mutation of genes encoding BMP inhibitors provided insights into how enhanced BMP signaling affects embryonic development, especially skeletal development, none of the mutant mouse lines generated by this strategy are useful for postnatal HO studies. This likely reflects the pleiotropic roles of BMP signaling in various tissues.

**2.3. Animal Models That Overexpress BMP Ligand.** The rationale for enhancing BMP signaling by overexpressing BMP ligand is straightforward, but this approach has met a number of unexpected complications. Overexpression of BMP4 under the control of many different promoters does not lead to postnatal HO. For example, HO does not develop after overexpression of BMP4 under control of either the keratin promoter (K14) [32] or the bovine cytokeratin IV promoter [56]. Transgenic mice overexpressing BMP4 under control of surfactant protein-C gene promoter die from abnormally formed lungs [57]. Transgenic mice expressing BMP4 in cartilage under the control of the *Col11a2* promoter/enhancer sequences die at birth due to respiratory failure [58] while mice overexpressing human BMP4 under control of mouse *Msx1* minimal promoter develop no visible abnormalities [33]. Overexpression of BMP2 under the human  $\alpha$ SM-actin promoter in an ApoE-deficient background accelerates atherosclerotic intimal calcification in transgenic lines but does not produce typical HO [59]. Mice that overexpress BMP4 under the *Nephrin* promoter have interesting defects in glomerular capillary formation but not the HO phenotype [60].

The only exception has been mice that overexpress BMP4 under control of the neuron specific enolase promoter (*Nse-BMP4*). These mice develop a phenotype that closely recapitulates the FOP phenotype and that also displays the histological hallmarks of typical acquired HO [34]. These findings suggest that overexpression of BMP itself may be necessary but is not sufficient to generate the HO phenotype and that the correct expression patterns or contexts are crucial. We have extensively characterized the phenotype in this transgenic mouse line and have used these mice, in collaboration with other labs [39], to study different aspects of HO, including definition of the events that trigger HO, the type of cells that respond to the trigger by differentiating along the osteogenic lineage, and the mechanisms underlying the spread of HO [61].

**2.4. Animal Models That Overexpress a Specific BMP Target Gene.** If overexpression of BMP ligand can produce HO, it is reasonable to think that expressing specific BMP target genes might also be capable of copying the phenotype of BMP overexpression. In fact, overexpression of *MSX2*, a BMP target gene, can induce an HO-like phenotype. *MSX2* overexpression elicited the phenotype under control of either an ubiquitous promoter, such as CMV, a tissue specific promoter, such as TIMP1 (tissue inhibitor of metalloproteinase 1), or the endogenous *MSX2* promoter [35, 62]. Overexpression of another BMP target gene, *Runx2*, under the type II collagen promoter also caused an HO-like phenotype and ectopic expression of hypertrophic chondrocyte markers [36]. These two models show that both *MSX2* and *Runx2* can partially mediate the osteogenic effects of BMPs in vivo. However, since the phenotypes in these two lines do not closely mimic that of FOP, the relevance of these models to the human disease is still unclear. Moreover, multiple transgenic lines that overexpress other BMP target genes, especially the *Id* family genes, that is, *Id1-Id4* [63–65], have failed to produce an HO phenotype. This could be partially explained by the inadequate tissue specific promoters used in generating these transgenic lines. However, the failure more likely indicates that not all BMP target genes are important in mediating the HO phenotype, even though *Id1* and *Id3* are positive factors in promotion of bone formation in vivo [65].

**2.5. Animal Models That Overexpress Other Factors That Indirectly Modify the BMP Signaling Pathway.** Theoretically, it is also possible to enhance the BMP signaling indirectly through factors that can interact with components of the BMP signaling pathway. For example, overexpression of *Fos* in bone cells under control of an FBJ long terminal repeat element (H2-FosLTR) resulted in the development of calcified tumors similar to HO, and *Fos-ES* cell chimeras developed chondrosarcomas with high efficiency at all skeletal sites containing cartilage [37]. However, transgenic mice that overexpress other related AP1 members (e.g., JUN and FOSB) do not exhibit abnormalities, despite high expression in bone tissue. Not surprisingly, further studies provided evidence of specific interactions between the BMP-signaling pathway and c-Fos, but not the other related AP1 members in FOP-like lesions [38].

Overall, even though there are multiple ways to enhance BMP signaling in vivo, only a few genetic modified animal lines showed typical HO, or a phenotype resembling FOP. Further, only one line, *Nse-BMP4* transgenic mice, closely recapitulated the major aspects of the FOP phenotype.

### 3. Animal Models for Acquired HO

Acquired HO usually follows traumatic events, such as fracture, total hip arthroplasty, muscular trauma, spinal cord injury, or central nervous system injury. It is a relatively frequent clinical complication with a wide clinical spectrum but normally it has a relatively benign course [12]. The etiology of common acquired HO is still unclear, and multiple contributing factors have been proposed including BMPs,

inflammation, prostaglandin E2, hypercalcemia, hypoxia, abnormal nerve activities, immobilization, and disequilibrium of hormones [66, 67]. Lack of deep understanding of underlying molecular mechanisms has directly hindered the validation of existing animal models, and this also has limited the development of new mechanism-based animal models. Currently, there are several available animal models that can produce typical HO: (1) heterotopic implantation models, (2) hip arthroplasty model, (3) the immobilization manipulation model (also called the Michelsson model), (4) Achilles tenotomy model, (5) trauma-induced model, and (6) models generated by injection of irritants and other materials to muscle.

**3.1. Heterotopic Implantation Model.** Currently the most commonly used animal model for HO involves the surgical implantation of BMP containing matrix at heterotopic sites. Implantation of demineralized bone matrix was first used by Urist in 1965 [68]; then Wozney et al. were able to repeat the experiment using partially purified BMP proteins [69]. Currently, the most widely used approach is BMP matrigel implantation [39]; an advantage of this method is that a chilled mix can be injected into heterotopic sites as a liquid which gels on site at body temperature and thereafter releases BMP4 continuously at the site. Many modifications/variations of this method have been used in different species under different conditions, including introduction of a DNA construct that produces BMPs [40], microbubble-enhanced transcuteaneous sonoporation of human BMP2 [70], nanogel-cross-linking hydrogel as a scaffold [41], implantation of a slow-release system of polylactic acid and rhBMP-2, or sintered porous-surfaced Ti-6Al-4V implants coated with native BMPs [71].

One interesting variation on this theme involves direct injection into the heterotopic site of cells that have osteogenic, and/or osteogenic factor producing potential, such as bone marrow cells [42], or implantation of a diffusion chamber containing such cells. Tested cell types have included urinary tract epithelia [72], certain transformed cells such as transformed human amnion cells (FL cells) [73], Moloney sarcoma [74], and epithelial-like cells [75]. In a similar system, these cells are impregnated into ceramic blocks to test their osteogenic activity [76] in the presence or absence of an osteogenic inducer.

Another interesting approach takes advantage of the osteoinductive ability of certain biomaterials, such as microporous calcium phosphate ceramic particles [43], that do not release BMP or other known osteogenic factors. The mechanism of osteoinduction by such biomaterials is not currently clear, although the geometry of the material is thought to be important [77].

Generally, heterotopic implantation models are straightforward, repeatable, and mechanistically relevant to human HO. However, certain limitations do exist: (1) they are artificial systems that may create unphysiologically high local concentrations of osteogenic factors in implanted sites leading to effects not relevant to the human disorder, (2) the implantation is a local event and thus has limited ability to mimic the potential effects of the involvement of multiple

systems, (3) different variations of this method have variable reliabilities and relevance to human conditions, (4) the incidence of implantation-induced bone formation varies depending upon the material or animal species. Normally rabbits are the most, and mice the least, susceptible [78], and experimental conditions that produce ectopic bone do not always coincide with clinical observations in humans.

**3.2. Hip Arthroplasty Model.** HO is commonly observed after hip arthroplasty in humans for unknown reasons. To develop a model relevant to the human condition [44], Schneider et al. subjected rabbits to surgery analogous to human hip arthroplasty either with or without muscle and bone injury on each hip. This led to HO, and the effectiveness of postoperative radiation in prophylaxis of HO was then analyzed using this model. The rationale behind this model is straightforward, and it can produce HO with certain reliability; however, despite being a phenocopy of the human condition, it is not a mechanism-based model. This method has not been widely adapted by other investigators, probably due to the relatively complicated surgical procedure.

**3.3. The Michelsson Model (Also Called Immobilization Manipulation Model).** Michelsson et al. [45] found that repeated forced mobilization of an immobilized knee joint caused HO in the quadriceps muscle in rabbits, and similar procedures can induce HO around other joints in the rabbit as well. The precise inductive stimulus has not been identified in this model, but an interaction between the periosteum and the necrotic muscle seems necessary since the introduction of a plastic membrane between bone and muscle prevents bone formation [79]. The first sign of osteoblastic activity was seen in the periosteum, and the new bone was often formed in continuity with the periosteum. Interestingly, early changes in prostaglandins preceded bone formation [80], consistent with the hypothesis that inflammation is the basis of the heterotopic bone formation in that process. Several authors have used this model to study the development and prevention of HO in animals [81–85]. However, since HO in this model is not affected by denervation, in contradistinction to clinical findings in patients with neurologic injuries, the relevance of this model to human HO is unclear.

**3.4. Achilles Tenotomy Model.** The Achilles tenotomy model was first described in rats by Buck in 1953 [46], and in 1983, McClure applied the model to mice and found that ectopic bone developed in 60% of animals by 5 weeks and in 100% by 10 weeks after Achilles tenotomy [86]. The advantages of this model are its relative simplicity and excellent predictability. However, the molecular mechanisms of HO induced by Achilles tenotomy are poorly understood, and the relevance of this model to clinical conditions is also unclear since ectopic bone formation in Achilles tendon is a rare condition in humans. Further, in humans HO is not only associated with prior surgery or trauma to the tendon but is also an important manifestation of rheumatoid arthritis and ankylosing spondylitis [86].

**3.5. Trauma-Induced Models.** Traumatic muscle or CNS injury often leads to HO in humans, but the underlying causative factor(s) remains unknown. Efforts to establish trauma induced models have had only limited success. Zaccalini and Urist failed to induce HO in rabbit thigh by blunt force [47]. Walton et al. reported limited success in inducing HO in sheep thigh by repeated blunt force (7 out of 42 sheep) [48]. Further, intramembranous and not endochondral ossification was the histological feature within scar tissue. Based on these reports, these models do not seem to be sufficiently reliable to be used routinely. Further, the failure of this strategy has forced us to rethink why trauma, which clearly plays a role in human HO, does not routinely induce it in such models. Fortunately recent studies using Nse-BMP4 mice have demonstrated that mild trauma leads to HO with high frequency irrespective of which limb is injured. In turn this suggests that trauma-induced HO depends upon susceptibility determined by other factors—in this case elevated levels of BMP4. The high frequency and reproducibility of trauma-induced HO in this model may provide a means of exploring the underlying mechanisms.

**3.6. Irritant and Other Miscellaneous Material-Induced Models.** Injection of various irritant materials into muscle sometimes leads to HO. For example, Heinen et al. reported the induction of HO in rabbit by injection of 40% ethanol [49]. Selle and Urist also reported that acid-alcohol could induce HO in a small percent of animals, while injections of calcium chloride produced only amorphous calcified plaques, not new bone or cartilage [50]. In addition, Arai et al. [87] and Caselli et al. [88] reported a controversial finding that colchicine induced intramedullary bone formation. This finding could not be repeated by K. H. Wlodarski and P. Wlodarski [89], and later Dudkiewicz et al. found that colchicine actually inhibits HO in a rabbit model [90]. The issues of repeatability and relevance of these models to human HO limits their potential utility.

Overall, due to limited understanding of molecular mechanisms, most animal models for acquired HO can only mimic some aspects of the human conditions. Further, the reliability and questionable clinical relevance hinder their use as drug test platforms. Thus caution must be taken in choosing one of these models to be appropriate for the specific question being asked.

## 4. Summary and Future Directions

Multiple animal models have been generated for studies of HO (see Table 1). For the simplicity of description in this review, we divided these models into two major groups, acquired or hereditary. However, to some extent, this division is arbitrary since injury and inflammation facilitates and triggers HO in FOP as well as in animal models of hereditary HO, and the high variability in susceptibility of different individuals to acquired HO suggests a genetic basis for individual predisposition. In fact, accumulating clinical and experimental evidence suggests that similar cellular and molecular mechanisms underlie the pathophysiology of all

TABLE 1: Summary of commonly used animal models.

	General strategy	examples	HO related phenotypes?	references
Animal models of hereditary HO	Hyperactive BMP receptor	ACVR1(R206H) mutation into zebrafish	No HO	[20]
		Conditionally expresses caALK2 in mice	Embryonic lethal, no HO	[29]
	Knocking out BMP inhibitors	Noggin-/- mice	Postnatal lethal, skeletal defects, HO?	[30]
		Chordin-/- mice	Postnatal lethal, skeletal defects	[31]
	Overexpressing BMPs	K14-BMP4	No HO	[32]
		Msx1-BMP4	No observable defects	[33]
		<b>Nse-BMP4</b>	<b>Progressive HO, FOP-like</b>	<b>[34]</b>
	Overexpressing BMP target genes	CMV-MSX2, TIMP1-MSX2 or MSX2-MSX2	HO-like	[35]
		col2-Runx2	HO-like	[36]
	Modifying BMP signaling indirectly	H2FosLTR-Fos	Calcified tumors similar to HO	[37]
Fos-ES cell chimeras		Chondrosarcomas, similar to HO	[38]	
Animal models of acquired HO	Heterotopic implantation	BMP matrigel implantation	HO	[39]
		DNA construct that produces BMPs	HO	[40]
		Nanogel-cross-linking hydrogel as a scaffold	HO	[41]
		Bone marrow cells	HO	[42]
	Hip arthroplasty model	Microporous calcium phosphate ceramic	HO	[43]
		Hip arthroplasty in rabbits	HO	[44]
	Michelsson model	Immobilization manipulation in rabbits	HO	[45]
	Achilles tenotomy model	Achilles tenotomy in rats	HO	[46]
	Trauma-induced model	Blunt force in rabbit thigh	No HO	[47]
		Repeated blunt force in sheep thigh	HO in small % of treated animals	[48]
Inject irritants and other materials	Injection of 40% ethanol	HO	[49]	
	Injection of acid-alcohol	HO in small % of treated animals	[50]	

typical HO which involves formation of fibroproliferative lesions containing cells that follow the classic endochondral ossification pathway. Thus, in hereditary HO, a specific genetic mutation plays the central role, while in acquired HO the environmental factors play more important roles. For this reason, some animal models such as Nse-BMP4 mice can be used to study both hereditary and acquired HO.

Understanding the fundamental pathophysiology underlying HO is the key to development of mechanism-based animal models. Just as determination of the genetic basis of FOP opened up a whole new avenue for generating models for hereditary HO, deeper understanding of the molecular

mechanisms underlying acquired HO will lead to more fruitful approaches in generating new animal models for the disorder. Multiple contributing factors are necessary for acquired HO including a trigger (trauma, injury), osteogenic progenitor cells, and a permissive microenvironment. However, thus far there is no single hypothesis that integrates most clinical and experimental findings, and current data strongly suggests the involvement of multiple organ systems in this disorder. For this reason, future multidisciplinary studies of neuroimmunological interactions and osteoneuroimmunology using currently available animal models, such as Nse-BMP4 mice, will be necessary to provide the

new insights which in turn could lay the foundation for new mechanism-based animal models.

## Acknowledgments

The authors thank Dr. Frederick Kaplan, who has been an inspiration to us and to the entire field, for his advice, encouragement, and support. This paper was supported in part by Grants to Lixin Kan from the Center for Research in FOP and Related disorders of the University of Pennsylvania School of Medicine. John A Kessler is supported by the NIH Grants nos. R01 020013-25 and R01 020778-25.

## References

- [1] H. C. Pape, S. Marsh, J. R. Morley, C. Krettek, and P. V. Giannoudis, "Current concepts in the development of heterotopic ossification," *Journal of Bone and Joint Surgery B*, vol. 86, no. 6, pp. 783–787, 2004.
- [2] D. E. Garland, "A clinical perspective on common forms of acquired heterotopic ossification," *Clinical Orthopaedics and Related Research*, no. 263, pp. 13–29, 1991.
- [3] S. Egli, J. Rodriguez, and R. Ganz, "Heterotopic ossification in total hip arthroplasty: the significance for clinical outcome," *Acta Orthopaedica Belgica*, vol. 66, no. 2, pp. 174–180, 2000.
- [4] O. S. Nilsson and P.-E. Persson, "Heterotopic bone formation after joint replacement," *Current Opinion in Rheumatology*, vol. 11, no. 2, pp. 127–131, 1999.
- [5] P. Slatis, O. Kiviluoto, and S. Santavirta, "Ectopic ossification after hip arthroplasty," *Annales Chirurgiae et Gynaecologiae*, vol. 67, no. 3, pp. 89–93, 1978.
- [6] C. A. Cipriano, S. G. Pill, and M. A. Keenan, "Heterotopic ossification following traumatic brain injury and spinal cord injury," *Journal of the American Academy of Orthopaedic Surgeons*, vol. 17, no. 11, pp. 689–697, 2009.
- [7] F. Genet, J.-L. Marmorat, C. Lautridou, A. Schnitzler, L. Mailhan, and P. Denormandie, "Impact of late surgical intervention on heterotopic ossification of the hip after traumatic neurological injury," *Journal of Bone and Joint Surgery B*, vol. 91, no. 11, pp. 1493–1498, 2009.
- [8] J. A. Forsberg and B. K. Potter, "Heterotopic ossification in wartime wounds," *Journal of Surgical Orthopaedic Advances*, vol. 19, no. 1, pp. 54–61, 2010.
- [9] J. A. Forsberg, J. M. Pepek, S. Wagner et al., "Heterotopic ossification in high-energy wartime extremity injuries: prevalence and risk factors," *Journal of Bone and Joint Surgery A*, vol. 91, no. 5, pp. 1084–1091, 2009.
- [10] E. M. Shore and F. S. Kaplan, "Insights from a rare genetic disorder of extra-skeletal bone formation, fibrodysplasia ossificans progressiva (FOP)," *Bone*, vol. 43, no. 3, pp. 427–433, 2008.
- [11] A. Dejerine and A. Ceillier, "Paraosteopathies of paraplegic patients by spinal cord lesion: clinical and roentgenographic study," *Clinical Orthopaedics and Related Research*, no. 263, pp. 3–12, 1991.
- [12] N. Cullen and J. Perera, "Heterotopic ossification: pharmacologic options," *Journal of Head Trauma Rehabilitation*, vol. 24, no. 1, pp. 69–71, 2009.
- [13] U. M. Carl and K. A. Hartmann, "Heterotopic calcification as a late radiation effect: report of 15 cases," *British Journal of Radiology*, vol. 75, no. 893, pp. 460–463, 2002.
- [14] V. D. Pellegrini Jr. and C. M. Evarts, "Radiation prophylaxis of heterotopic bone formation following total hip arthroplasty: current status," *Seminars in Arthroplasty*, vol. 3, no. 3, pp. 156–166, 1992.
- [15] F. S. Kaplan and E. M. Shore, "Perspective: progressive osseous heteroplasia," *Journal of Bone and Mineral Research*, vol. 15, no. 11, pp. 2084–2094, 2000.
- [16] H. Jüppner, "The genetic basis of progressive osseous heteroplasia," *New England Journal of Medicine*, vol. 346, no. 2, pp. 128–130, 2002.
- [17] F. Atzeni, P. Sarzi-Puttini, and M. Bevilacqua, "Calcium deposition and associated chronic diseases (atherosclerosis, diffuse idiopathic skeletal hyperostosis, and others)," *Rheumatic Disease Clinics of North America*, vol. 32, no. 2, pp. 413–426, 2006.
- [18] G. Feldman, M. Li, S. Martin et al., "Fibrodysplasia ossificans progressiva, a heritable disorder of severe heterotopic ossification, maps to human chromosome 4q27-31," *American Journal of Human Genetics*, vol. 66, no. 1, pp. 128–135, 2000.
- [19] E. M. Shore, M. Xu, G. J. Feldman, D. A. Fenstermacher, M. A. Brown, and F. S. Kaplan, "A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva," *Nature Genetics*, vol. 38, no. 5, pp. 525–527, 2006.
- [20] Q. Shen, S. C. Little, M. Xu et al., "The fibrodysplasia ossificans progressiva R206H ACVR1 mutation activates BMP-independent chondrogenesis and zebrafish embryo ventralization," *Journal of Clinical Investigation*, vol. 119, no. 11, pp. 3462–3471, 2009.
- [21] T. Fukuda, M. Kohda, K. Kanomata et al., "Constitutively activated ALK2 and increased SMAD1/5 cooperatively induce bone morphogenetic protein signaling in fibrodysplasia ossificans progressiva," *Journal of Biological Chemistry*, vol. 284, no. 11, pp. 7149–7156, 2009.
- [22] A. Yabuzoe, S.-I. Yokoi, M. Sekiguchi et al., "Fibrodysplasia ossificans progressiva in a Maine Coon cat with prominent ossification in dorsal muscle," *Journal of Veterinary Medical Science*, vol. 71, no. 12, pp. 1649–1652, 2009.
- [23] K. Asano, A. Sakata, H. Shibuya et al., "Fibrodysplasia ossificans progressiva-like condition in a cat," *Journal of Veterinary Medical Science*, vol. 68, no. 9, pp. 1003–1006, 2006.
- [24] B. A. Valentine, C. George, J. F. Randolph, S. A. Center, L. Fuhrer, and K. A. Beck, "Fibrodysplasia ossificans progressiva in the cat. A case report," *Journal of Veterinary Internal Medicine*, vol. 6, no. 6, pp. 335–340, 1992.
- [25] H. B. Warren and J. L. Carpenter, "Fibrodysplasia ossificans in three cats," *Veterinary Pathology*, vol. 21, no. 5, pp. 495–499, 1984.
- [26] M. J. Guilliard, "Fibrodysplasia ossificans in a German shepherd dog," *Journal of Small Animal Practice*, vol. 42, no. 11, pp. 550–553, 2001.
- [27] H. R. Seibold and C. L. Davis, "Generalized myositis ossificans (familial) in pigs," *Pathologia veterinaria*, vol. 4, no. 1, pp. 79–88, 1967.
- [28] B. Rothschild, D. Larry, L. D. Martin, and R. M. Timm, "A new spontaneous model of fibrodysplasia ossificans progressiva," *Nature Precedings*, 2008.
- [29] T. Fukuda, G. Scott, Y. Komatsu et al., "Generation of a mouse with conditionally activated signaling through the BMP receptor, ALK2," *Genesis*, vol. 44, no. 4, pp. 159–167, 2006.
- [30] P. Tylzanowski, L. Mebis, and F. P. Luyte, "The Noggin null mouse phenotype is strain dependent and haploinsufficiency leads to skeletal defects," *Developmental Dynamics*, vol. 235, no. 6, pp. 1599–1607, 2006.
- [31] D. Bachiller, J. Klingensmith, N. Shneyder et al., "The role of chordin/Bmp signals mammalian pharyngeal development

- and DiGeorge syndrome," *Development*, vol. 130, no. 15, pp. 3567–3578, 2003.
- [32] U. Guha, W. A. Gomes, T. Kobayashi, R. G. Pestell, and J. A. Kessler, "In vivo evidence that BMP signaling is necessary for apoptosis in the mouse limb," *Developmental Biology*, vol. 249, no. 1, pp. 108–120, 2002.
- [33] X. Zhao, Z. Zhang, Y. Song et al., "Transgenically ectopic expression of Bmp4 to the Msx1 mutant dental mesenchyme restores downstream gene expression but represses Shh and Bmp2 in the enamel knot of wild type tooth germ," *Mechanisms of Development*, vol. 99, no. 1-2, pp. 29–38, 2000.
- [34] L. Kan, M. Hu, W. A. Gomes, and J. A. Kessler, "Transgenic mice overexpressing BMP4 develop a fibrodysplasia ossificans progressiva (FOP)-like phenotype," *American Journal of Pathology*, vol. 165, no. 4, pp. 1107–1115, 2004.
- [35] Y. H. Liu, R. Kundu, L. Wu et al., "Premature suture closure and ectopic cranial bone in mice expressing Msx2 transgenes in the developing skull," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 13, pp. 6137–6141, 1995.
- [36] C. Ueta, M. Iwamoto, N. Kanatani et al., "Skeletal malformations caused by overexpression of Cbfa1 or its dominant negative form in chondrocytes," *Journal of Cell Biology*, vol. 153, no. 1, pp. 87–100, 2001.
- [37] Z.-Q. Wang, A. E. Grigoriadis, U. Mohle-Steinlein, and E. F. Wagner, "A novel target cell for c-fos-induced oncogenesis: development of chondrogenic tumours in embryonic stem cell chimeras," *EMBO Journal*, vol. 10, no. 9, pp. 2437–2450, 1991.
- [38] E. A. Olmsted, F. H. Gannon, Z.-Q. Wang et al., "Embryonic overexpression of the c-Fos protooncogene: a murine stem cell chimera applicable to the study of fibrodysplasia ossificans progressiva in humans," *Clinical Orthopaedics and Related Research*, no. 346, pp. 81–94, 1998.
- [39] V. Y. Lounev, R. Ramachandran, M. N. Wosczyzna et al., "Identification of progenitor cells that contribute to heterotopic skeletogenesis," *The Journal of Bone and Joint Surgery. American volume*, vol. 91, no. 3, pp. 652–663, 2009.
- [40] H. Volek-Smith and M. R. Urist, "Recombinant human bone morphogenetic protein (rhBMP) induced heterotopic bone development in vivo and in vitro," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 211, no. 3, pp. 265–272, 1996.
- [41] C. Hayashi, U. Hasegawa, Y. Saita et al., "Osteoblastic bone formation is induced by using nanogel-crosslinking hydrogel as novel scaffold for bone growth factor," *Journal of Cellular Physiology*, vol. 220, no. 1, pp. 1–7, 2009.
- [42] A. J. Friedenstein, I. I. Piatetzky-Shapiro, and K. V. Petrakova, "Osteogenesis in transplants of bone marrow cells," *Journal of Embryology and Experimental Morphology*, vol. 16, no. 3, pp. 381–390, 1966.
- [43] D. Le Nihouannen, G. Daculsi, A. Saffarzadeh et al., "Ectopic bone formation by microporous calcium phosphate ceramic particles in sheep muscles," *Bone*, vol. 36, no. 6, pp. 1086–1093, 2005.
- [44] D. J. Schneider, M. J. R. Moulton, K. Singapuri et al., "The Frank Stinchfield Award. Inhibition of heterotopic ossification with radiation therapy in an animal model," *Clinical Orthopaedics and Related Research*, no. 355, pp. 35–46, 1998.
- [45] J.-E. Michelsson, G. Granroth, and L. C. Andersson, "Myositis ossificans following forcible manipulation of the leg. A rabbit model for the study of heterotopic bone formation," *Journal of Bone and Joint Surgery A*, vol. 62, no. 5, pp. 811–815, 1980.
- [46] R. C. Buck, "Regeneration of tendon," *Journal of Pathology & Bacteriology*, vol. 66, no. 1, pp. 1–18, 1953.
- [47] P. S. Zaccalini and M. R. Urist, "Traumatic periosteal proliferations in rabbits. The enigma of experimental myositis ossificans traumatica," *The Journal of Trauma*, vol. 4, pp. 344–357, 1964.
- [48] M. Walton and A. G. Rothwell, "Reactions of thigh tissues of sheep to blunt trauma," *Clinical Orthopaedics and Related Research*, vol. 176, pp. 273–281, 1983.
- [49] J. H. Heinen Jr., G. H. Dabbs, and H. A. Mason, "The experimental production of ectopic cartilage and bone in the muscles of rabbits," *The Journal of Bone and Joint Surgery. American volume*, vol. 31A, no. 4, pp. 765–775, 1949.
- [50] R. W. Selle and M. R. Urist, "Calcium deposits and new bone formation in muscle in rabbits," *Journal of Surgical Research*, vol. 1, pp. 132–141, 1961.
- [51] T. Kobayashi, K. M. Lyons, A. P. McMahon, and H. M. Kronenberg, "BMP signaling stimulates cellular differentiation at multiple steps during cartilage development," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 50, pp. 18023–18027, 2005.
- [52] R. J. Wordinger, G. Zode, and A. F. Clark, "Focus on molecules: gremlin," *Experimental Eye Research*, vol. 87, no. 2, pp. 78–79, 2008.
- [53] E. Gaggero, A. Smerdel-Ramoya, S. Zanotti et al., "Conditional deletion of gremlin causes a transient increase in bone formation and bone mass," *Journal of Biological Chemistry*, vol. 282, no. 43, pp. 31549–31557, 2007.
- [54] M. S. Dionne, W. C. Skarnes, and R. M. Harland, "Mutation and analysis of Dan, the founding member of the Dan family of transforming growth factor  $\beta$  antagonists," *Molecular and Cellular Biology*, vol. 21, no. 2, pp. 636–643, 2001.
- [55] E. H. Simpson, D. K. Johnson, P. Hunsicker, R. Suffolk, S. A. Jordan, and I. J. Jackson, "The mouse Cer1 (Cerberus related or homologue) gene is not required for anterior pattern formation," *Developmental Biology*, vol. 213, no. 1, pp. 202–206, 1999.
- [56] M. Blessing, L. B. Nanney, L. E. King, C. M. Jones, and B. L. M. Hogan, "Transgenic mice as a model to study the role of TGF- $\beta$ -related molecules in hair follicles," *Genes and Development*, vol. 7, no. 2, pp. 204–215, 1993.
- [57] S. Bellusci, R. Henderson, G. Winnier, T. Oikawa, and B. L. M. Hogan, "Evidence from normal expression and targeted misexpression that Bone Morphogenetic Protein-4 (Bmp-4) plays a role in mouse embryonic lung morphogenesis," *Development*, vol. 122, no. 6, pp. 1693–1702, 1996.
- [58] N. Tsumaki, T. Nakase, T. Miyaji et al., "Bone morphogenetic protein signals are required for cartilage formation and differently regulate joint development during skeletogenesis," *Journal of Bone and Mineral Research*, vol. 17, no. 5, pp. 898–906, 2002.
- [59] Y. Nakagawa, K. Ikeda, Y. Akakabe et al., "Paracrine osteogenic signals via bone morphogenetic protein-2 accelerate the atherosclerotic intimal calcification in vivo," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 30, pp. 1908–1915, 2010.
- [60] H. Ueda, Y. Miyazaki, T. Matsusaka et al., "Bmp in podocytes is essential for normal glomerular capillary formation," *Journal of the American Society of Nephrology*, vol. 19, no. 4, pp. 685–694, 2008.
- [61] L. Kan, Y. Liu, T. L. McGuire et al., "Dysregulation of local stem/progenitor cells as a common cellular mechanism for heterotopic ossification," *Stem Cells*, vol. 27, no. 1, pp. 150–156, 2009.

- [62] Y. H. Liu, M. L. Snead, and R. E. Maxson Jr., "Transgenic mouse models of craniofacial disorders," *Methods in Molecular Biology*, vol. 137, pp. 499–512, 2000.
- [63] M. A. Morrow, E. W. Mayer, C. A. Perez, M. Adlam, and G. Siu, "Overexpression of the Helix-Loop-Helix protein Id2 blocks T cell development at multiple stages," *Molecular Immunology*, vol. 36, no. 8, pp. 491–503, 1999.
- [64] B. M. Wice and J. I. Gordon, "Forced expression of Id-1 in the adult mouse small intestinal epithelium is associated with development of adenomas," *Journal of Biological Chemistry*, vol. 273, no. 39, pp. 25310–25319, 1998.
- [65] Y. Maeda, K. Tsuji, A. Nifuji, and M. Noda, "Inhibitory helix-loop-helix transcription factors Id1/Id3 promote bone formation in vivo," *Journal of Cellular Biochemistry*, vol. 93, no. 2, pp. 337–344, 2004.
- [66] E. F. McCarthy and M. Sundaram, "Heterotopic ossification: a review," *Skeletal Radiology*, vol. 34, no. 10, pp. 609–619, 2005.
- [67] L. V. Bossche and G. Vanderstraeten, "Heterotopic ossification: a review," *Journal of Rehabilitation Medicine*, vol. 37, no. 3, pp. 129–136, 2005.
- [68] M. R. Urist, "Bone: formation by autoinduction," *Science*, vol. 150, no. 3698, pp. 893–899, 1965.
- [69] J. M. Wozney, V. Rosen, A. J. Celeste et al., "Novel regulators of bone formation: molecular clones and activities," *Science*, vol. 242, no. 4885, pp. 1528–1534, 1988.
- [70] K. Osawa, Y. Okubo, K. Nakao, N. Koyama, and K. Bessho, "Osteoinduction by microbubble-enhanced transcutaneous sonoporation of human bone morphogenetic protein-2," *Journal of Gene Medicine*, vol. 11, no. 7, pp. 633–641, 2009.
- [71] Z. Simon, D. A. Deporter, R. M. Pilliar, and C. M. Clokie, "Heterotopic bone formation around sintered porous-surfaced Ti-6Al-4V implants coated with native bone morphogenetic proteins," *Implant Dentistry*, vol. 15, no. 3, pp. 265–274, 2006.
- [72] C. B. Huggins, "The phosphatase activity of transplants of the epithelium of the urinary bladder to the abdominal wall producing heterotopic ossification," *Biochemical Journal*, vol. 25, no. 3, pp. 728–732, 1931.
- [73] H. C. Anderson, "Electron microscopic studies of induced cartilage development and calcification," *Journal of Cell Biology*, vol. 35, no. 1, pp. 81–101, 1967.
- [74] K. Wlodarski and J. Thyberg, "Demonstration of virus particles in Moloney murine sarcoma virus-induced periosteal bone in mice," *Virchows Archiv Abteilung B Cell Pathology*, vol. 46, no. 1-2, pp. 109–117, 1984.
- [75] K. Wlodarski, "Induction of heterotopic and orthotopic cartilage and bone formation in mice," *Acta Biologica Hungarica*, vol. 35, no. 2–4, pp. 205–218, 1984.
- [76] H. Ohgushi, V. M. Goldberg, and A. I. Caplan, "Heterotopic osteogenesis in porous ceramics induced by marrow cells," *Journal of Orthopaedic Research*, vol. 7, no. 4, pp. 568–578, 1989.
- [77] H. Yuan, Z. Yang, J. D. De Bruijn, K. De Groot, and X. Zhang, "Material-dependent bone induction by calcium phosphate ceramics: a 2.5-year study in dog," *Biomaterials*, vol. 22, no. 19, pp. 2617–2623, 2001.
- [78] F. Feldman, "Soft tissue mineralization: roentgen analysis," *Current Problems in Diagnostic Radiology*, vol. 15, no. 3, pp. 161–240, 1986.
- [79] J.-E. Michelsson, M. Pettila, T. Valtakari, I. Leivo, and H. J. Aho, "Isolation of bone from muscles prevents the development of experimental callus-like heterotopic bone: a study of the interaction of bone and muscle in new bone formation," *Clinical Orthopaedics and Related Research*, no. 302, pp. 266–272, 1994.
- [80] C. S. Bartlett, B. E. Rapuano, D. G. Lorch et al., "Early changes in prostaglandins precede bone formation in a rabbit model of heterotopic ossification," *Bone*, vol. 38, no. 3, pp. 322–332, 2006.
- [81] L. C. Vanden Bossche, G. Van Maele, I. Wojtowicz et al., "Free radical scavengers versus methylprednisolone in the prevention of experimentally induced heterotopic ossification," *Journal of Orthopaedic Research*, vol. 27, no. 6, pp. 748–751, 2009.
- [82] P. G. Tsailas, G. C. Babis, K. Nikolopoulos, P. N. Soucacos, and D. S. Korres, "The effectiveness of two COX-2 inhibitors in the prophylaxis against heterotopic new bone formation: an experimental study in rabbits," *Journal of Surgical Research*, vol. 151, no. 1, pp. 108–114, 2009.
- [83] J. R. Hardy and P. Rooney, "Use of the myositis ossificans model of Michelsson," *Clinical orthopaedics and related research*, no. 336, pp. 340–342, 1997.
- [84] B. R. Moed, R. B. Resnick, A. J. Fakhouri, B. Nallamothu, and R. A. Wagner, "Effect of two nonsteroidal antiinflammatory drugs on heterotopic bone formation in a rabbit model," *Journal of Arthroplasty*, vol. 9, no. 1, pp. 81–87, 1994.
- [85] H. J. Aho, H. Aro, S. Juntunen, L. Strengell, and J.-E. Michelsson, "Bone formation in experimental myositis ossificans. Light and electron microscopic study," *APMIS*, vol. 96, no. 10, pp. 933–940, 1988.
- [86] J. McClure, "The effect of diphosphonates on heterotopic ossification in regenerating Achilles tendon of the mouse," *Journal of Pathology*, vol. 139, no. 4, pp. 419–430, 1983.
- [87] N. Arai, K. Ohya, and H. Ogura, "Osteopontin mRNA expression during bone resorption: an in situ hybridization study of induced ectopic bone in the rat," *Bone and Mineral*, vol. 22, no. 2, pp. 129–145, 1993.
- [88] G. Caselli, S. Fiorentino, M. Riminucci, A. Corsi, and P. Bianco, "Does colchicine really induce bone formation in the rodent bone marrow? Yes, it does," *Calcified Tissue International*, vol. 65, no. 5, pp. 414–415, 1999.
- [89] K. H. Wlodarski and P. Wlodarski, "Colchicine-induced osteogenesis: demonstration versus proof," *Calcified Tissue International*, vol. 69, no. 1, pp. 58–59, 2001.
- [90] I. Dudkiewicz, I. Cohen, S. Horowitz et al., "Colchicine inhibits heterotopic ossification: experimental study in rabbits," *Israel Medical Association Journal*, vol. 7, no. 1, pp. 31–34, 2005.