White Rose Research Online

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/188632/

Version: Published Version

**Article:**
Felix-Ilemhenbhio, F., Pickering, G.A.E., Kiss-Toth, E. et al. (2022) Pathophysiology and emerging molecular therapeutic targets in heterotopic ossification. International Journal of Molecular Sciences, 23 (13). 6983. ISSN: 1422-0067

https://doi.org/10.3390/ijms23136983

**Reuse**
This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here: https://creativecommons.org/licenses/

**Takedown**
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

International Journal of
*Molecular Sciences*

MDPI

*Review*

# Pathophysiology and Emerging Molecular Therapeutic Targets in Heterotopic Ossification

**Favour Felix-Ilemhenbhio [1,2], George A. E. Pickering [1], Endre Kiss-Toth [2] and Jeremy Mark Wilkinson [1,*]**

[1] Department of Oncology and Metabolism, The Medical School, University of Sheffield, Beech Hill Road, Sheffield S10 2RX, UK; ffelixilemhenbhio1@sheffield.ac.uk (F.F.-I.); georgeaepickering@gmail.com (G.A.E.P.)
[2] Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield S10 2RX, UK; e.kiss-toth@sheffield.ac.uk
* Correspondence: j.m.wilkinson@sheffield.ac.uk

**Abstract:** The term heterotopic ossification (HO) describes bone formation in tissues where bone is normally not present. Musculoskeletal trauma induces signalling events that in turn trigger cells, probably of mesenchymal origin, to differentiate into bone. The aetiology of HO includes extremely rare but severe, generalised and fatal monogenic forms of the disease; and as a common complex disorder in response to musculoskeletal, neurological or burn trauma. The resulting bone forms through a combination of endochondral and intramembranous ossification, depending on the aetiology, initiating stimulus and affected tissue. Given the heterogeneity of the disease, many cell types and biological pathways have been studied in efforts to find effective therapeutic strategies for the disorder. Cells of mesenchymal, haematopoietic and neuroectodermal lineages have all been implicated in the pathogenesis of HO, and the emerging dominant signalling pathways are thought to occur through the bone morphogenetic proteins (BMP), mammalian target of rapamycin (mTOR), and retinoic acid receptor pathways. Increased understanding of these disease mechanisms has resulted in the emergence of several novel investigational therapeutic avenues, including palovarotene and other retinoic acid receptor agonists and activin A inhibitors that target both canonical and non-canonical signalling downstream of the BMP type 1 receptor. In this article we aim to illustrate the key cellular and molecular mechanisms involved in the pathogenesis of HO and outline recent advances in emerging molecular therapies to treat and prevent HO that have had early success in the monogenic disease and are currently being explored in the common complex forms of HO.

**Keywords:** heterotopic ossification; genetics; bone morphogenetic protein; activin A/ALK2; retinoic acid receptor; Hoxa11+ mesenchymal stromal cells

check for updates

**Citation:** Felix-Ilemhenbhio, F.; Pickering, G.A.E.; Kiss-Toth, E.; Wilkinson, J.M. Pathophysiology and Emerging Molecular Therapeutic Targets in Heterotopic Ossification. *Int. J. Mol. Sci.* **2022**, *23*, 6983. https://doi.org/10.3390/ijms23136983

Academic Editor: José Manuel López

Received: 26 May 2022
Accepted: 22 June 2022
Published: 23 June 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

## 1. Introduction

Heterotopic ossification (HO) is a disorder characterised by bone development within tissues where bone does not normally exist. Several presentations of HO have been described since its early documentation in 1883 by Riedel and its first association with musculoskeletal trauma in World War One combatants in 1918 [1]. There are two forms of HO traditionally described: the rare 'genetic disease', and the more common acquired, or 'post-traumatic', HO. The monogenic HO diseases, which follow a Mendelian pattern of inheritance, include fibrodysplasia ossificans progressiva (FOP) and progressive ossific heteroplasia (POH). FOP is a rare debilitating disease with a prevalence of 1–2 cases per million persons in which muscle and connective tissues are gradually substituted by bone that is commonly triggered by minor trauma events [2,3]. POH is an extremely rare disease affecting less than 60 people worldwide, ref. [4,5] in which ossification develops initially in the deeper layers of the dermis and subcutaneous fat and spreads to include muscle and tendons as the disease progresses. Both diseases are associated with progressive disability and early death [6]. The term acquired, or "post-traumatic" HO describes extra-skeletal

bone formation that occurs following musculoskeletal or neurological trauma and burns [7]. Acquired HO occurs in 20–30% of patients with spinal cord injury [8], 10–20% of patients with closed head injury [8], up to 50% of patients after total hip replacement [9], and up to 70% of patients following high-energy combat trauma [10].

The present review provides an overview of our current understanding of the molecular biology of HO initiation and development, including the cellular and genetic origins of HO. Based on these molecular advances in our understanding of the disease, we also review the current status of evolving molecular therapies for HO prevention and treatment. Throughout the article, we use the term HO to describe acquired HO and the terms FOP and POH to describe the specific monogenic disorders.

## 2. Overview of Normal Bone Formation

In order to understand the mechanisms of bone formation in HO, a brief review of normal bone formation is given against which HO development will be compared. Normal mature bone is formed through one of two mechanisms, termed intramembranous and endochondral ossification. The progenitor cell for both processes is the mesenchymal precursor, but the mechanism and site at which ossification occurs differs (reviewed in [11,12]). In intramembranous ossification, a sheet of mesenchymal connective tissue, termed the fibrous membrane, forms the template of the future bone. Bones forming through this mechanism are typically flat, including the cranium, sternum, ribs, and scapula. The mesenchymal precursor cells differentiate into osteoblasts or into supporting blood vessels. The osteoblasts secrete osteoid, an extracellular matrix comprising collagen and other organic proteins that entraps the osteoblasts as the osteoid mineralises. Once entrapped, the osteoblasts trans-differentiate into osteocytes that remain as mechanosensing cells within the bone matrix. Osteoblasts on the surface of the bone transdifferentiate to form a cellular layer termed the periosteum. The periosteum is responsible for cortical bone synthesis, and envelopes the cancellous bone that is continuous with the haematopoietic red bone marrow. In endochondral ossification, bone formation occurs through an intermediate, cartilaginous stage that serves as a template for the final bone. The long bones, including the clavicle, humerus, radius, ulna, metacarpals, phalanges, femur, fibula, tibia, metatarsals, and phalanges form through endochondral ossification. The process commences as mesenchymal stem cells condense and differentiate into chondrocytes to form the cartilage template. This is followed by hypertrophy and subsequent apoptosis of the central cells, whilst mesenchymal progenitors at the template surface differentiate into osteoblasts and osteoclasts. The hypertrophic and apoptotic cartilage core is innervated, vascularised, and replaced by bone and bone marrow in the primary ossification centre. At the developing bone metaphysis, a hypertrophic component of the growing cartilage is constantly substituted by trabecular bone to mediate longitudinal bone growth. The non-vascularised cartilage at the ends of the bone is invaded by epiphyseal vessels to initiate the secondary ossification centre. Between the epiphyseal and metaphyseal bone centres reside layers of chondrocytes that form growth plates to further support longitudinal growth. Longitudinal growth stops as the growth plate is fully resorbed to leave a single marrow cavity within the long bone.

## 3. Cellular Origins of HO

In HO development, following the initiating stimulus bone may form within a range of extraosseous tissues of mesenchymal origin and may involve either of the above mechanisms of bone formation. The architectural features of HO resemble normal bone and include a zonal mineralization pattern, mature cortical bone at the periphery, and a central marrow component [13]. Chalmers et al. (1975) first proposed the basic common requirements for HO formation: osteogenic precursor cells, a permissive environment and an inducing agent [14]. This model is consistent with HO formation through either the endochondral or intramembranous routes. A summary of current concepts of the cellular origins of HO is given below and is reviewed further elsewhere [15,16].

### 3.1. Hematopoietic Cells

In the 1970s, hematopoietic stem cells or other precursors recruited to the lesions from bone marrow were suggested to contribute to the induction and formation of ectopic bone in patients with FOP [17]. Lymphocytes taken from FOP patients were subsequently shown to overexpress Bone Morphogenetic Protein 4 (BMP4), a potent bone-inducing protein [18]. Olmsted-Davis and colleagues investigated hematopoietic side-population (SP) cells as possible precursors for HO [19]. These cells were known to possess multi-lineage potential, with the ability to differentiate into skeletal myocytes [20] and vascular endothelial cells [21]. SP cells were isolated from the bone marrow of C57BL/6 CD45.2 Rosa26 mice and their osteogenic potential was tested by transplantation into C57BL/6 CD45.1 mice. Both osteoblasts and osteocytes from the subsequent newly formed bone stained positively for markers of donors SP cells indicating osteogenic potential [19]. Dominici and colleagues demonstrated in Friend leukemia virus B/ NIH Jackson (FVB/NJ) mice that transplantable fluorescently-labelled marrow cells from the non-adherent population can produce functional osteoblasts, osteocytes and hematopoietic cells [22]. Kaplan and colleagues observed in a patient with FOP that bone marrow transplantation for treating anaemia was not sufficient to inhibit FOP, but that pharmacological suppression of the donor's immune system following transplantation inhibited FOP [23]. In mice, hematopoietic cells contributed to the inflammatory and bone marrow-repopulating stages of BMP4-induced HO by recruiting and activating osteogenic precursors, but they did not act directly as a cellular precursor of HO [23]. These findings contrast with those of Otsuru and colleagues who showed a contribution of hematopoietic cells to bone formation in BMP2-induced intramuscular HO, although only a minority of bone-marrow derived cells were embedded in the definitive heterotopic bone [24,25]. More recently, analysis of clinical tissue following musculoskeletal injury in humans has demonstrated that circulating osteogenic progenitor cells of bone marrow origin, characterised by both type 1 collagen and CD45 immunopositivity, are found in early fibroproliferative and neovascular HO lesions, supporting the concept that circulating mononuclear progenitors can seed inflammatory sites to initiate HO formation [26]. Taken together these studies suggest that haematopoietic cells of bone marrow origin contribute to both FOP and acquired HO development most likely through their creation of the necessary pro-osteogenic environment, but are unlikely to be significant direct osteogenic progenitors.

### 3.2. Endothelial Cells

Vascular endothelial cells have been suggested as a primary candidate for HO formation due to their multilineage potential via endothelial-mesenchymal transition (EndMT) and the expression of endothelial markers in FOP lesions [27,28]. During EndMT, endothelial cells loose cell-cell adhesion and change polarity, reconfiguring into a spindle-shape, and reducing the expression of endothelial markers whilst increasing mesenchymal marker expression. Following transition, these cells are highly motile and invasive and play an important role in both tissue development and disease [29–31]. Medici and colleagues showed in vitro that endothelial cells over-expressing Activin Receptor-like Kinase 2 (ALK2, also called ACRV1), or treated with the ALK2 ligands TGF-β2 or BMP4, can dedifferentiate into stem cells with the capacity to re-differentiate into cartilage or bone cells [28]. In vivo data in the neuron-specific enolase-BMP4 (NSE-BMP4) mouse also show that ectopic cartilage and bone cells express endothelial biomarkers such as vWF, VE-cadherin, Tie1, and Tie2 after injection of purified BMP [27,28], after transgenic over-expression of ALK2 [28], or after muscle injury [27]. Tie2 and vWF are also expressed in chondrogenic and osteogenic lesions from FOP patients, whereas osteoblasts and chondrocytes from normal cartilage or bone do not express these biomarkers [28]. Lineage tracing in Tie2-Cre transgenic mice found that 50% of the cartilage and bone cells in HO lesions were of endothelial origin [27,28]. However, CD31+ endothelial cells were shown to not contribute to heterotopic cartilage or bone formation directly in the mouse following intramuscular BMP2 injection, but they did participate in lesion angiogenesis [32] and to HO development following burn/tenotomy

injury [33]. The different outcomes in the last two studies may be attributed to differences in the Cre drivers or in the HO models used [16]. A further limitation of the lineage trace studies is that the markers expressed by endothelial cells can also be expressed by other cell types. Tie2, which is expressed in endothelial cells to regulate development and maintenance of vasculature [34], is also expressed in hematopoietic cells [35,36], and by a population of Tie2+ PDGFRα+ Sca1+ multipotent mesenchymal progenitors that was shown to contribute to HO initiation [32]. Furthermore, musculoskeletal injury induces expression of endothelial markers Tie2, CD31 and VE-cadherin in mesenchymal, non-endothelial cells [33]. Taking together, the studies outlined above suggest that endothelial cells can undergo EndMT to initiate HO but they are unlikely to be pivotal, as Tie2+, CD31+ or VeCadherin+ progenitors also arise from other cell types and vary with the HO induction model used. These inconsistencies underscore the idea that the cellular populations contributing to HO development are highly tissue and context-specific.

### 3.3. Fibro-Adipogenic Cells

Fibro-adipogenic precursors (FAPs) are a population of PDGFRα+ SCA1+ multipotent cells located within, but not exclusive to, skeletal muscle [32,37,38]. FAPs are found near vascular elements, but are unlike pericytes in that they do not share a basal lamina with the endothelium and are NG2- [32,38]. Muscle resident FAPs support muscle regeneration but lack myogenic potential [29,37,38]. FAPs were first discovered due to their fibrogenic and adipogenic capacities [37,38]. They were later shown to possess osteogenic potential when stimulated with BMP in culture and in vivo [32]. Wosczyna and colleagues observed that Tie2-Cre lineage labelled FAPs made up ~50% of heterotopic bone and cartilage in the mouse [32]. These cells have been proposed to play a major role in human FOP [39,40]. Several studies in the mouse show that progenitors of intramuscular and intratendinous HO are frequently PDGFRα+ and positive for cartilage and bone formation markers [38,39,41–44]. Using a mouse FOP model in which *ACVR1* (that encodes ALK2) was genetically manipulated, Dey and colleagues showed that FAP-like cells can be divided into two lineages, Scx+ tendon-derived progenitors and a muscle-resident interstitial Mx1+ population [39]. The Scx+ progenitors mediated endochondral HO without exogenous injury, whilst the Mx1+ population mediated injury-dependent HO. PDGFRα+ cells made up a minor subgroup of Mx1+ and Scx+ lineages; however, constitutive activation of *ACVR1* signalling demonstrated that PDGFRα+ subsets had an enhanced osteogenic and chondrogenic potential compared to unfractionated Scx+ or Mx1+ cells. Eisner and colleagues demonstrated that tissue resident FAPs in skeletal muscle are the primary source of osteogenic cells in the murine BMP2-Matrigel model of post-traumatic HO [44]. In the same study using Notexin to induce muscle damage, they demonstrated that FAPs contribute to the formation of mature bone without the addition of exogenous BMP2. Moreover, when FAPs were cleared by macrophages at day 3–4 after injury, osteogenic genes were downregulated. Taken together, these findings suggest that FAPs can contribute to most HO presentations due to their broad distribution across tissue types and their documented presence in HO and that cells of hematopoietic origin play a role in stimulating their osteogenic potential.

### 3.4. Myosatellite Cells

Myosatellite cells are myogenic muscle-resident stem cells that are pivotal in skeletal muscle regeneration [45]. They are located between the myofibre sarcolemma and basal lamina, and give rise to myodifferentiated cells following muscle injury [46]. They were initially considered a primary precursor for muscle HO due to their osteogenic potential in culture in response to BMPs in the C2C12 murine myoblast model [47] and in human myogenic progenitor cells [48]. However, lineage and transplantation studies indicate that they contribute minimally to BMP-induced HO in vivo [27,32,49,50]. Further, targeted expression of constitutively-activated *ACVR1/ALK2* (caACVR1) [39,51] and *ACVR1* (R206H) [40] in myosatellite cells is insufficient to induce HO. Although Lees-Shephard and Goldhamer [16] have proposed that myosatellite cells do not contribute to HO initiation,

several lines of in vivo data do support their role in its pathogenesis. BMP signalling is a primary mechanism leading to the formation of acquired and genetic HO and has also been associated with the physiological regulation of skeletal muscle mass [52]. When transplanted into the quadriceps of nude mice, skeletal muscle myoblasts have been shown to promote osteogenic differentiation [53]. Muscle-derived stem cells express BMP4 and differentiate into bone [54]. BMPs at the location of muscle injury inhibit myogenesis and promote osteogenesis of myoblasts, both in vitro [47] and in vivo [32]. Further, serum taken from animals following a burn injury increases the osteogenic capacity of myosatellite cells, suggesting a role in burn-induced HO [55]. Taken together, these findings indicate that the muscle tissue provides a permissive environment for HO and that following musculoskeletal trauma BMPs can modulate endogenous muscle progenitors to form heterotopic bone.

### 3.5. Other Cell Types

Through in vivo models, several other progenitor cell types have also been identified, including pericytes, tendon and ligament progenitors, and transient brown adipocyte-like cells (Table 1). Although these cell types are associated with HO initiation, their precise contributions remain unclear. More recently, using a burn/tenotomy injury in *Hoxa11-CreER$^{T2}$*; *ROSA-LSL-TdTomato* mice, Pagani and colleagues have traced the cell fate of MSCs in HO development using single-cell sequencing [56]. They found that MSCs of the *Hoxa11* lineage differentiate through both the endochondral and osteogenic route into HO bone in the mouse forelimb following burn/tenotomy injury. During HO progression, the *Hoxa11*-lineage cells expressed transcriptional profiles characteristic of both osteogenesis and chondrogenesis. Previous studies have shown that *Hoxa11*+ multipotent stromal cells are self-renewing and persistent throughout the life of mice, and that Hoxa11 contributes to bone formation, maintenance and repair [57–59].

**Table 1.** Overview of cell types investigated for their contribution to heterotopic ossification.

| Cell Type | Location | Description | Key Papers |
| --- | --- | --- | --- |
| Hematopoietic cells | Bone marrow | Contribute to inflammation and marrow-repopulating stages. Contribution to HO is unclear. | [19,23,25,60] |
| Endothelial cells | Blood and lymphatic vessels | Contribute to HO through EndMT route, but may be overestimated due to lack of surface marker endothelial cell-specificity. | [28,35] |
| FAPs | Muscle and related soft tissues; widely spread in other tissues | Support muscle regeneration. Contribute to a high percentage of HO. | [32,43,61] |
| Myosatellite cells | Muscle | BMP2-induced HO. Contribution low based on most lineage studies. | [32,48] |
| Pericytes | Vascular basement membrane | BMP-induced HO but assessment of contribution unclear due to high degree of heterogeneity. | [50,62–64] |
| Hoxa11+ Mesenchymal stromal cells | Tendon, muscle and skeletal tissue | Contribute to skeletal repair, express chondrogenic and osteogenic transcription profile following injury. | [56–59] |
| Tendon and ligament progenitor cells | Tendon<br>Ligament | Account for 25 and 40% of heterotopic bone and cartilage, respectively, after bone/tendonectomy based on Scx-Cre labelling. Molecularly heterogeneous. | [39,43,65] |

**Table 1.** *Cont.*

| Cell Type | Location | Description | Key Papers |
| --- | --- | --- | --- |
| Sensory neurons | Dermis, epidermis, and muscle spindle | Mediate HO formation via substance P and calcitonin gene-related peptide. BMP2 may induce neurogenic inflammation to remodel nerve and release HO precursor cells. May explain how HO occurs following traumatic brain injury. Mice lacking sensory neurons cells do not develop HO. Tie2+ endoneurial progenitors the major HO cell contributors in a mice model; however, Tie2 marker is also expressed in endothelial and mesenchymal cells. | [66–69] |
| Transient brown adipocyte-like cells | Adipose | Specialized pool of brown adipocytes that contribute to HO. Associated to deposition of cartilage. Detected in human traumatic injury-induced HO. | [70,71] |

Due to the heterogenic nature of HO aetiology, several cell types contribute depending on the site and initiating factors. This raises the issue of which cell and experimental model is most appropriate for investigating the function of HO susceptibility genes in culture and/or in vivo. A conclusive answer to this question remains elusive, nevertheless, the role of specific genes may be best examined by investigating how they affect the signalling response of precursor cells to promote bone formation and/or maintenance using an experimental model most appropriate to the type of HO investigated.

## 4. Signalling Pathways in HO

### *4.1. BMP Signalling*

BMPs are a family of signalling molecules that belong to the Transforming Growth Factor-β (TGF-β) superfamily of proteins. Discovered by Urist in 1965 [72], they play a crucial role in bone formation and repair, and in HO development [73]. During normal bone development and physiological homeostasis, BMP ligands bind to a heterotetrameric complex of two BMPRI and two BMPRII transmembrane serine/threonine kinase receptors to initiate chondrogenesis and osteogenesis. The BMPs that initiate signalling through this mechanism and the osteogenic processes that they initiate are summarised in Table 2.

**Table 2.** Overview of BMPs and their role in major cellular process and heterotopic ossification.

| Signalling Protein | Function | Key Papers |
| --- | --- | --- |
| BMP1 | Bone formation and homeostasis. | [74] |
| BMP2 | Induces bone and cartilage development. Induces EndMT transition. Also involved in hedgehog pathway, cardiac cell differentiation, embryonic development. | [75–78] |
| BMP3 | Bone and cartilage development; antagonizes other BMPs in osteo-differentiation. | [79] |
| BMP4 | Potently induces chondro- and osteogenic differentiation; induces EndMT transition. Also involved in embryonic development, adipogenesis, neurogenesis. | [80–83] |
| BMP5 | Bone and cartilage development; may play a role in some cancer types; expressed in the visual apparatus. | [84–86] |

**Table 2.** *Cont.*

| Signalling Protein | Function | Key Papers |
| --- | --- | --- |
| BMP6 | Osteogenic differentiation; closely related to BMP5 and BMP7; regulates iron metabolism | [87–89] |
| BMP7 | Bone homeostasis; induces osteoblast differentiation through SMAD canonical pathway; involved in embryonic development, adipogenesis. | [90–92] |
| BMP8 | Expressed in developing skeleton; osteogenesis and germ cell generation. | [93–96] |
| BMP9/GDF2 | Induces chondro- and osteogenesis; cannot be blocked by BMP3 unlike most BMPs; involved in lymphatic development. | [97–99] |
| BMP10 | Involved in the trabeculation oof the heart and regulates monocyte recruitment to the vascular endothelium. | [100–102] |
| BMP11/GDF11 | Augments bone formation; induces embryonic development. | [103,104] |
| BMP12/GDF7 | Inhibits endochondral bone growth; induces tenogenic differentiation; regulates bone structure | [105] |
| BMP13/GDF6/CDMP2 | Establishes the boundaries between skeletal elements during development; induces tenogenic differentiation | [105,106] |
| BMP14/GDF5/CDMP1 | Regulates skeletal development and joint formation; promotes fracture healing. | [106–108] |
| BMP15 | Involved in fertilization and ovulation | [109,110] |

Four type I BMP receptors (ALK1, ALK2 (also termed ACVR1), ALK3 and ALK6) bind BMP ligands. Three receptors (BMPR2, ALK4 and ALK7) serve as type II BMP receptors. ALK4 and ALK7 (also termed ActR-IIA and ActR-IIB), also act as receptors for activins, whilst BMPR2 only binds BMPs (Figure 1).

Downstream signalling following BMP receptor activation occurs through 2 distinct pathways: 1. SMAD canonical pathways, in which SMAD 1/5/8 proteins are phosphorylated to promote expression of chondro- or osteogenic genes [111]; 2. Non-canonical SMAD pathways where p38 MAPK, ERK or JNK are activated [112–115]. Under normal physiological conditions, these chondro- and osteogenic signalling pathways are antagonised by Activin A (another TGF-β superfamily member) binding to a heterotetrameric receptor complex comprising two ActR BMPRII receptors and two BMPRI receptors to initiate SMAD2/3 phosphorylation and downstream signalling as a negative feedback mechanism for gene transcriptional activation that is initiated by BMP signalling [116]. These pathways should not be viewed as independent, as crosstalk between them occurs [117–120]. BMP2 is overexpressed in clinically evolving HO tissue after trauma [121,122]. Augmented BMP signalling also occurs following trauma-induced HO development in animal models whilst BMP antagonism reduces HO severity [123,124]. Experimental models of HO therefore commonly use exogenous BMP2 [27,68] or overexpression of BMP4 [125], or recombinant BMP2 (rhBMP2) [27,126] as the HO initiator. BMP signalling is also a key feature of the heritable forms of the disease [127]. In FOP, a mutation in *ACVR1* that encodes the BMP type 1 receptor ALK2, causes its constitutive activation, initiating downstream BMP signalling regardless of BMP ligand binding [124].

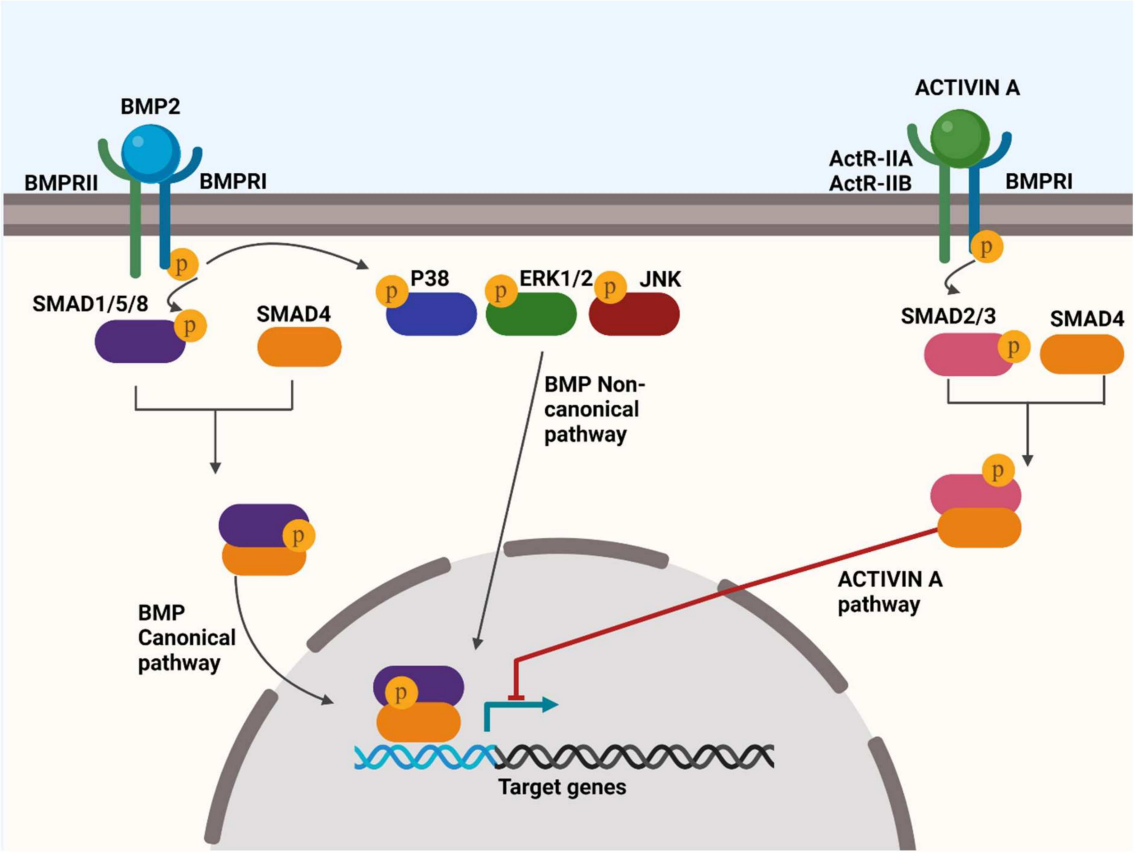


**Figure 1.** BMP receptor activation and downstream signalling and its antagonism through the Activin A pathway. In the canonical pathway, SMAD1/5/8 is activated and interacts with SMAD4 to promote expression of target genes that induce bone formation. In the non-canonical SMAD pathway, p38 MAPK, ERK1/2 and/or JNK are activated to promote the expression of osteogenic target genes. BMP signalling is antagonised by the binding of Activin A to its receptor complex to initiate SMAD2/3 signalling that acts to suppress BMP target gene transcriptional activation.

### *4.2. mTOR Signalling*

The mammalian target of rapamycin (mTOR) signalling pathway is involved in several cellular processes, including chondrogenesis, osteogenesis and skeletal development [128,129]. The FOP activating mutation in *ACVR1* has been shown to increase mTOR signalling [130]. Conversely, rapamycin suppresses bone formation in experimental models for FOP [41,130], trauma-induced HO [41,131], and in leptin-induced osteogenesis in both in vitro and in vivo models [132] through inhibition of mTOR complexes mTORC1 and mTORC2 [133] (Figure 2). Rapamycin is currently being studied in a phase 2 clinical trial (UMIN000028429) of the disease. BMP2 also promotes osteogenesis through an mTORC1-dependent mechanism [134], whilst mTORC2 modulates osteogenesis in response to a range of mechanical or chemical cues [128,135,136].

### *4.3. Other Signalling Pathways*

Hypoxia-inducible factors (HIFs) activate genes that mediate adaptive responses to reduced oxygen tension [137,138]. HIFs augment HO formation [41] and couple bone and vascular growth during development [138]. Retinoic acid receptor (RAR) signalling is mediated by retinoids (metabolic derivatives of vitamin A), which are potent morphogens that promote both chondro- and osteogenesis to shape skeletal development [139]. In retinoic acid (RA) mediated gene activation, RA binds to a heterodimer complex comprising RAR and the retinoid X receptor (RAR-RXR). RAR-RXR then activates gene transcription by binding to DNA motifs termed RA-response elements (RARE) located within enhancer

regions of RA target genes [140]. In the absence of RA, unliganded RAR-RXR recruits histone deacetylases and nuclear corepressors to inhibit transcriptional activation at the RARE [140,141]. Chondrogenesis requires the absence of RA signalling, in which the repressor function of unliganded RAR-RXR on RAREs dominates [141,142], whilst active RA signalling prevents the chondrogenic differentiation of precursor cells [143]. Crosstalk between the HIF and RAR signalling systems is well documented, but how they co-operate to modulate bone formation is still incompletely understood [144–148]. Due to the pleiotropic function of these pathways, it is anticipated that any therapeutic application to inhibit HO may have off-target effects, as these pathways also dynamically regulate several other critical cellular processes [149].

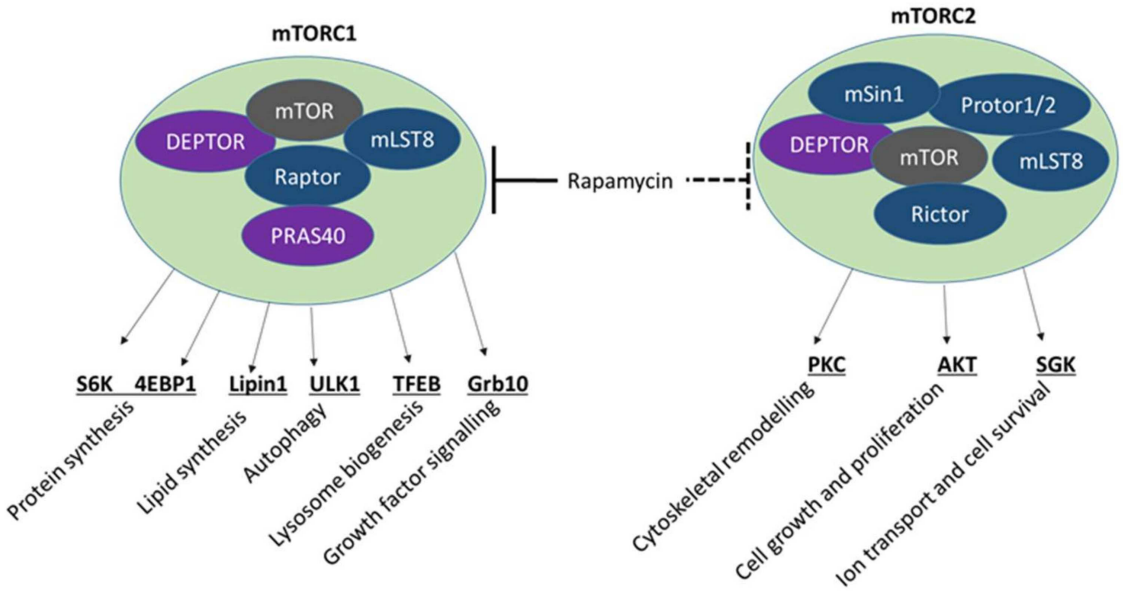


**Figure 2.** mTOR signalling pathway. Rapamycin inhibits mTORC1 and mTORC2, which in turn modulate several downstream osteogenic pathways. Acute rapamycin treatment inhibits mTORC1 whilst repeated dosing of rapamycin also inhibits mTORC2. Both mTORC1 and mTORC2 are activated by Wnt and IGF. mTORC1 is also activated by BMP2 and mTORC2 is also activated by mechanical and chemical signals to promote osteogenesis.

## 5. Therapeutic Strategies for HO

Treatment strategies for acquired HO to date have included the use of anti-inflammatory agents, bisphosphonates, local radiation therapy, and surgical resection. Systematic reviews have shown that patients treated with either selective or non-selective non-steroidal anti-inflammatory drugs (NSAIDs) showed a significant decrease in post-traumatic HO formation when compared with placebo [150–152], but were associated with a higher rate of drug discontinuation due to gastrointestinal side effects. Low-dose local radiation therapy also decreases the incidence of HO after surgery [153,154], but carries the risk of irradiation-induced malignancy [155] and side-effects such as delayed wound-healing, progressive soft-tissue contracture, non-union, and inhibited ingrowth of cementless hip implants [156,157]. The treatment of mature HO after trauma involves surgical resection, although complete excision may not be feasible and recurrence is common [158–160]). Simple bisphosphonates, such as etidronate, have also been studied as a prophylactic intervention in HO, as they delay matrix mineralisation. However, bisphosphonates do not inhibit bone matrix synthesis, and mineralisation recommences after drug discontinuation [161,162]. None of these strategies specifically target molecular pathways involved in HO pathogenesis. However, as our understanding of these cells and pathways evolves, molecular mechanism-specific investigative therapeutic approaches are beginning to emerge, as outlined below).

### 5.1. Palovarotene and Other RAR Agonists

The observation that RA signalling suppresses chondrogenesis has stimulated its investigation as a therapeutic target for HO. Synthetic retinoid agonists selective for nuclear RARα or RARγ have been tested in mouse models of injury-induced intramuscular HO, implantation of rhBMP-2 and constitutive activation of mutant *ACVR1* (Q207D) [51,163]. Whilst RA agonists targeting both RARα and RARγ inhibited endochondral HO, those targetting RARγ were most effective as RARγ is more strongly and selectively expressed in chondrogenic cells than other RAR members [164,165]. Chakkalakal and colleagues showed that palovarotene prevented HO, restored long bone growth, and preserved growth plate function in transgenic mice carrying the human *ACVR1* (R206H) mutation for classic FOP [166]. In juvenile FOP mice, palovarotene reduced HO both in vitro and in vivo, but resulted in aggressive synovial joint overgrowth and long bone growth plate ablation [167]. In a rat model of post-traumatic HO (in which rats were subjected to blast overpressure via a shock tube resulting in femur fracture, soft tissue crush injury, and amputation through the zone of injury [168]), Palovarotene treatment suppressed the systemic and local inflammatory response, decreased osteogenic progenitor colonies by 98% in both in vitro and in vivo, and decreased the expression of osteo-and chondrogenic genes, including BMP4 [168]. In another trauma-induced model, rats were subjected to blast-related limb injury, femoral fracture, quadriceps crush injury, amputation and infection with methicillin-resistant Staphylococcus aureus (MRSA) [169]. Palovarotene treatment decreased HO by 50–60%, however 63% of rats treated with palovarotene and inoculated with MRSA experienced delayed healing or dehiscence compared to 25% of MRSA rats in the placebo arm of the study. Palovarotene is currently the subject of several clinical trials of efficacy and safety for the prevention of new HO lesions in both children and adults with FOP (www.clinicaltrials.gov; accessed on 5 May 2022, NCT02190747, NCT03312634, NCT02979769, NCT02521792, NCT05027802). However, whether Palovarotene or other RAR agonists represent a viable approach for treating acquired HO in humans remains unstudied.

### 5.2. Targeting ACVR1/ALK2 and Other Related Signalling Pathways

Under physiological conditions in normal tissues, the ligand Activin A interacts with ALK2 to mediate SMAD2/3 phosphorylation to regulate cell proliferation, apoptosis, and differentiation (Figure 1) [170–174]. In ALK2R206H+ FOP cells (that carry the common *ACVR1* mutation) ALK2 is activated constitutively in the absence of BMPs, enhancing both canonical and non-canonical BMP signalling pathways [175–179] to augment chondrogenesis [39,179–183]. Although the *ACVR1* mutation is not implicated in other forms of HO, ALK2 signalling has been explored as an investigational target due to its BMP agonism [184]. Table 3 provides a summary of molecular targets and investigational therapeutic strategies explored to date in HO prevention and treatment.

**Table 3.** Summary of investigational therapeutic strategies for the inhibition of heterotopic ossification, based on ALK2 signalling and other pathways. FOP = fibrodysplasia Ossificans Progressiva, tHO = acquired post-traumatic Heterotopic Ossification.

| Type of HO Pathways | Type of Molecule | Molecule | Description and Function | Key Papers |
|---|---|---|---|---|
| FOP | Antibody | REGN2477 (Garetosmab) | Anti-activin-A human monoclonal antibody in phase 2 clinical trial for FOP (LUMINA-1 study, NCT03188666). Blocks signalling of activin A, AB, and AC. Inhibits HO in animal model of FOP. | [179,185–187] |
| FOP | Antibody | Perhexiline maleate (Pex) | Identified in screening of 1040 FDA-approved drugs for suppression of the Id1 promoter activated by mutant ACVR1/ALK2 in mouse C2C12 myoblasts. Pex reduced HO volume in BMP-induced mouse model, but failed to inhibit HO in an open-label clinical trial in FOP. | [188,189] |
| tHO | Antibody | Metformin | Regulates osteogenic differentiation via AMPK, and RUNX2/CBFA1 in vitro and in vivo. Prevents traumatic HO in mouse by decreasing ALK2 and AMPK regulation of Smad2. | [190–192] |

**Table 3.** *Cont.*

| Type of HO Pathways | Type of Molecule | Molecule | Description and Function | Key Papers |
|---|---|---|---|---|
| FOP | Alpha-2 blocker | Fendiline hydrochloride | Identified in screen of 1040 FDA-approved drugs for suppression of the Id1 promoter activated by mutant ACVR1/ALK2. Mice administered with fendiline showed a slight reduction in HO. | [188] |
| FOP | Small molecule inhibitor | Dorsomorphin | Identified by chemical library screen for small molecules that dorsalise zebrafish embryos. Selectively inhibited ALK2 to block BMP-mediated SMAD1/5/8 phosphorylation. Preclinical use precluded by the inhibition of other ALKs (ALK3 and ALK6) and other kinases. | [176,193] |
| FOP, tHO | Small molecule inhibitor | LDN-193189 | An optimised version of dorsomorphin with greater potency and selectivity. Inhibits transcriptional activity of ALK2, ALK3, and constitutively active ALK2 mutant proteins. | [124] |
| FOP, tHO | Small molecule inhibitor | LDN-212854 | Derivative of dorsomorphin with increased selectivity for ALK2. LDN-212854 and LDN-193189 reduce osteogenic differentiation of tissue-resident MPCs from injured tissue following burn or tenotomy insult in animal model. In a blast-induced rat tHO model, LDN193189 and LDN212854 effective at limiting tHO. | [194,195] |
| FOP, tHO | Small molecule inhibitor | Other dorsomorphin derivatives | Currently undergoing investigation, including K02288, DMH-1, ML347, LDN 214117 and VU465350. | [196–198] |
| FOP | Small-molecule inhibitor | Saracatinib (AZD-0530) | Identified by screening compounds in an ALK2-mutated chondrogenic ATDC5 cell line. Inhibited both BMP and TGF-β signalling in vivo. Currently undergoing phase 2 clinical trial for FOP (NCT04307953). Well tolerated and potently inhibits the development of HO in inducible ALKQ207D transgenic and ACVR1R206H knock-in mouse. | [199–202] |
| FOP | Small-molecule inhibitor | PD 161570 | Identified by screening compounds in an ALK2-mutated chondrogenic ATDC5 cell line. Inhibits both BMP and TGF-β signalling in vivo. | [199] |
| FOP | Small-molecule inhibitor | TAK 165 | Identified by screening compounds in an ALK2-mutated chondrogenic ATDC5 cell line. Indirectly modulates mTOR signalling in vivo. | [199] |
| FOP | Ligand traps | sActR-IIA-Fc and sActR-IIB-Fc | ACVR1-Fc fusion proteins comprising the extracellular domain of human WT ACVR1 and the Fc portion of human immunoglobulin γ1. Inhibits dysregulated BMP signalling caused by FOP mutant ACVR1 and abrogates chondro-osseous differentiation in vitro. | [203–205] |
| FOP | Platelet inhibitor | Dipyridamole | Identified in screening of 1280 FDA-approved compounds for suppression of ACVR1 gene expression. Showed the highest inhibitory effect on SMAD signalling, chondrogenic and osteogenic differentiation in vitro. Reduced HO in BMP-induced model in mice. | [206,207] |
| FOP, tHO | Nucleotides | microRNAs | Altered expression of miRNA detected in HO. mir148b and mir365 down-regulate ACVR1/Alk-2 expression, whereas mir26a showed a positive effect on its mRNA. Inhibition of miRNAs, miR-146b-5p and -424 suppresses osteocyte maturation. Manipulating miR-574-3p levels both in vitro and in vivo inhibits chondrogenesis. miR-630 downregulated in early HO and used to distinguish HO from other processes in tHO. miR-17-5p upregulated in ankylosing spondylitis (AS) patients versus non-AS individuals. Knockdown and overexpression of miR-17-5p in fibroblasts derived from AS patients modulates osteogenesis. | [208–214] |
| FOP, tHO | Nucleotides | Antisense oligonucleotide (AON) | AON binds to specific exons in the primary mRNA transcript to prevent splicing and enable the skipping of specific exons. AONs designed to knockdown ALK2 expression in mice impair ALK2 signalling in both C2C12 end endothelial cells. However, AON affects both wild-type and mutated allele. | [215–217] |
| FOP, tHO | Nucleotides | RNA interference (RNAi) | Allele-specific siRNA (ASP-RNAi) duplexes tested for specific inhibition of mutant c.617A allele in mesenchymal progenitor cells from FOP patients. ASP-RNAi decreased BMP signalling to control cell levels. | [218,219] |
| tHO | Nucleotides | LncRNAs | Several lncRNAs regulate bone formation. Downregulation of *MANCR* inhibits osteoinduction in vitro. In a mouse in vivo tHO model, *Brd4-Mancr* signalling attenuated HO. | [220–222] |

## 6. Conclusions

In summary, heterotopic ossification may arise from both rare, heritable and common complex diseases. The downstream molecular pathways that underpin these heterogeneous aetiologies are broadly similar in both patterns of disease, although the diseases differ in extent and severity. Whether genetic or acquired, initiation of a new HO lesion involves tissue injury that results in a signal to initiate endochondral or intra-membranous ossification. The dominant cell types in HO include are FAPs, endothelial cells, hematopoietic cells, tendon and ligament progenitor cells, pericytes and Hoxa11+ mesenchymal stromal cells. The dominant pathways in HO include BMP, mTOR and RAR signalling. Several therapeutic strategies have been developed to target these signalling pathways. RAR agonists have been shown to be effective in preventing HO in pre-clinical models. Although the RAR agonist Palovarotene is undergoing clinical trials for FOP, further pre-clinical animal studies will be required to investigate its efficacy and safety for the post-traumatic HO indication. Several strategies have been developed to target ACVR1/ALK2 with REGN2477, metformin and dorsomorphin derivatives being a few prospects for clinical therapeutic applications. These future studies would benefit from translational experimental approaches that incorporate clinically relevant animal models in parallel with clinical investigations, population epidemiology studies and relevant molecular medicine techniques.

**Author Contributions:** Conceptualization, J.M.W. and E.K.-T.; Methodology, F.F.-I. and G.A.E.P.; Formal Analysis, F.F.-I.; Data Curation, F.F.-I.; Writing—Original Draft Preparation, F.F.-I. and G.A.E.P.; Writing—Review & Editing, J.M.W. and E.K.-T.; Visualization, F.F.-I.; Supervision, J.M.W. and E.K.-T.; Funding Acquisition, J.M.W. All authors have read and agreed to the published version of the manuscript.

**Funding:** Favour Felix-Ilemhenbhio was funded by a PhD Studentship from the Medical Research Council Versus Arthritis Centre for Integrated Research into Musculoskeletal Ageing, grant number MR/R502182/1. George Pickering was funded by a charitable studentship from the Cavendish Hip Foundation.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Dejerine, A.; Ceillier, A. Paraosteoarthropathies of paraplegic patients by spinal cord lesion. Clinical and roentgenographic study. *Clin. Orthop. Relat. Res.* **1991**, *263*, 3–12. [CrossRef]
2. Pignolo, R.J.; Hsiao, E.C.; Baujat, G.; Lapidus, D.; Sherman, A.; Kaplan, F.S. Prevalence of fibrodysplasia ossificans progressiva (FOP) in the United States: Estimate from three treatment centers and a patient organization. *Orphanet J. Rare Dis.* **2021**, *16*, 350. [CrossRef] [PubMed]
3. Shore, E.M.; Kaplan, F.S. Inherited human diseases of heterotopic bone formation. *Nat. Rev. Rheumatol.* **2010**, *6*, 518–527. [CrossRef] [PubMed]
4. Kaplan, F.S.; Craver, R.; MacEwen, G.D.; Gannon, F.H.; Finkel, G.; Hahn, G.; Tabas, J.; Gardner, R.J.; Zasloff, M.A. Progressive osseous heteroplasia: A distinct developmental disorder of heterotopic ossification. Two new case reports and follow-up of three previously reported cases. *J. Bone Jt. Surg. Am.* **1994**, *76*, 425–436. [CrossRef]
5. Kaplan, F.S.; Shore, E.M. Progressive osseous heteroplasia. *J. Bone Miner. Res.* **2000**, *15*, 2084–2094. [CrossRef] [PubMed]
6. Kaplan, F.S.; Zasloff, M.A.; Kitterman, J.A.; Shore, E.M.; Hong, C.C.; Rocke, D.M. Early mortality and cardiorespiratory failure in patients with fibrodysplasia ossificans progressiva. *J. Bone Jt. Surg. Am.* **2010**, *92*, 686–691. [CrossRef]
7. Vanden Bossche, L.; Vanderstraeten, G. Heterotopic ossification: A review. *J. Rehabil. Med.* **2005**, *37*, 129–136. [CrossRef]
8. Cipriano, C.A.; Pill, S.G.; Keenan, M.A. Heterotopic ossification following traumatic brain injury and spinal cord injury. *J. Am. Acad. Orthop. Surg.* **2009**, *17*, 689–697. [CrossRef]
9. Brooker, A.F.; Bowerman, J.W.; Robinson, R.A.; Riley, L.H., Jr. Ectopic ossification following total hip arthroplasty. Incidence and method of classification. *J. Bone Jt. Surg. Am.* **1973**, *55*, 1629–1632. [CrossRef]

10. Potter, B.K.; Burns, T.C.; Lacap, A.P.; Granville, R.R.; Gajewski, D.A. Heterotopic ossification following traumatic and combat-related amputations. Prevalence, risk factors, and preliminary results of excision. *J. Bone Jt. Surg. Am.* **2007**, *89*, 476–486. [CrossRef]
11. Berendsen, A.D.; Olsen, B.R. Bone development. *Bone* **2015**, *80*, 14–18. [CrossRef] [PubMed]
12. Buck, D.W., 2nd; Dumanian, G.A. Bone biology and physiology: Part I. The fundamentals. *Plast. Reconstr. Surg.* **2012**, *129*, 1314–1320. [CrossRef] [PubMed]
13. Shehab, D.; Elgazzar, A.H.; Collier, B.D. Heterotopic ossification. *J. Nucl. Med.* **2002**, *43*, 346–353. [PubMed]
14. Chalmers, J.; Gray, D.H.; Rush, J. Observations on the induction of bone in soft tissues. *J. Bone Jt. Surg. Br* **1975**, *57*, 36–45. [CrossRef]
15. Kan, L.; Kessler, J.A. Evaluation of the cellular origins of heterotopic ossification. *Orthopedics* **2014**, *37*, 329–340. [CrossRef]
16. Lees-Shepard, J.B.; Goldhamer, D.J. Stem cells and heterotopic ossification: Lessons from animal models. *Bone* **2018**, *109*, 178–186. [CrossRef]
17. Friedenstein, A.Y.; Lalykina, K.S. Lymphoid cell populations are competent systems for induced osteogenesis. *Calcif. Tissue Res.* **1970**, *4*, 105–106. [CrossRef]
18. Shafritz, A.B.; Shore, E.M.; Gannon, F.H.; Zasloff, M.A.; Taub, R.; Muenke, M.; Kaplan, F.S. Overexpression of an osteogenic morphogen in fibrodysplasia ossificans progressiva. *N. Engl. J. Med.* **1996**, *335*, 555–561. [CrossRef]
19. Olmsted-Davis, E.A.; Gugala, Z.; Camargo, F.; Gannon, F.H.; Jackson, K.; Kienstra, K.A.; Shine, H.D.; Lindsey, R.W.; Hirschi, K.K.; Goodell, M.A.; et al. Primitive adult hematopoietic stem cells can function as osteoblast precursors. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15877–15882. [CrossRef]
20. Gussoni, E.; Soneoka, Y.; Strickland, C.D.; Buzney, E.A.; Khan, M.K.; Flint, A.F.; Kunkel, L.M.; Mulligan, R.C. Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* **1999**, *401*, 390–394. [CrossRef]
21. Jackson, K.A.; Majka, S.M.; Wang, H.; Pocius, J.; Hartley, C.J.; Majesky, M.W.; Entman, M.L.; Michael, L.H.; Hirschi, K.K.; Goodell, M.A. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J. Clin. Investig.* **2001**, *107*, 1395–1402. [CrossRef] [PubMed]
22. Dominici, M.; Pritchard, C.; Garlits, J.E.; Hofmann, T.J.; Persons, D.A.; Horwitz, E.M. Hematopoietic cells and osteoblasts are derived from a common marrow progenitor after bone marrow transplantation. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11761–11766. [CrossRef] [PubMed]
23. Kaplan, F.S.; Glaser, D.L.; Shore, E.M.; Pignolo, R.J.; Xu, M.; Zhang, Y.; Senitzer, D.; Forman, S.J.; Emerson, S.G. Hematopoietic stem-cell contribution to ectopic skeletogenesis. *J. Bone Jt. Surg. Am.* **2007**, *89*, 347–357. [CrossRef]
24. Otsuru, S.; Tamai, K.; Yamazaki, T.; Yoshikawa, H.; Kaneda, Y. Bone marrow-derived osteoblast progenitor cells in circulating blood contribute to ectopic bone formation in mice. *Biochem. Biophys. Res. Commun.* **2007**, *354*, 453–458. [CrossRef]
25. Otsuru, S.; Tamai, K.; Yamazaki, T.; Yoshikawa, H.; Kaneda, Y. Circulating bone marrow-derived osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4/stromal cell-derived factor-1 pathway. *Stem Cells* **2008**, *26*, 223–234. [CrossRef]
26. Egan, K.P.; Duque, G.; Keenan, M.A.; Pignolo, R.J. Circulating osteogentic precursor cells in non-hereditary heterotopic ossification. *Bone* **2018**, *109*, 61–64. [CrossRef]
27. Lounev, V.Y.; Ramachandran, R.; Wosczyna, M.N.; Yamamoto, M.; Maidment, A.D.; Shore, E.M.; Glaser, D.L.; Goldhamer, D.J.; Kaplan, F.S. Identification of progenitor cells that contribute to heterotopic skeletogenesis. *J. Bone Jt. Surg. Am.* **2009**, *91*, 652–663. [CrossRef]
28. Medici, D.; Shore, E.M.; Lounev, V.Y.; Kaplan, F.S.; Kalluri, R.; Olsen, B.R. Conversion of vascular endothelial cells into multipotent stem-like cells. *Nat. Med.* **2010**, *16*, 1400–1406. [CrossRef]
29. De Angelis, L.; Berghella, L.; Coletta, M.; Lattanzi, L.; Zanchi, M.; Cusella-De Angelis, M.G.; Ponzetto, C.; Cossu, G. Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration. *J. Cell Biol.* **1999**, *147*, 869–878. [CrossRef]
30. Medici, D.; Kalluri, R. Endothelial-mesenchymal transition and its contribution to the emergence of stem cell phenotype. *Semin. Cancer Biol.* **2012**, *22*, 379–384. [CrossRef]
31. Potenta, S.; Zeisberg, E.; Kalluri, R. The role of endothelial-to-mesenchymal transition in cancer progression. *Br. J. Cancer* **2008**, *99*, 1375–1379. [CrossRef] [PubMed]
32. Wosczyna, M.N.; Biswas, A.A.; Cogswell, C.A.; Goldhamer, D.J. Multipotent progenitors resident in the skeletal muscle interstitium exhibit robust BMP-dependent osteogenic activity and mediate heterotopic ossification. *J. Bone Miner. Res.* **2012**, *27*, 1004–1017. [CrossRef] [PubMed]
33. Agarwal, S.; Loder, S.; Cholok, D.; Peterson, J.; Li, J.; Fireman, D.; Breuler, C.; Hsieh, H.S.; Ranganathan, K.; Hwang, C.; et al. Local and Circulating Endothelial Cells Undergo Endothelial to Mesenchymal Transition (EndMT) in Response to Musculoskeletal Injury. *Sci. Rep.* **2016**, *6*, 32514. [CrossRef] [PubMed]
34. Chu, M.; Li, T.; Shen, B.; Cao, X.; Zhong, H.; Zhang, L.; Zhou, F.; Ma, W.; Jiang, H.; Xie, P.; et al. Angiopoietin receptor Tie2 is required for vein specification and maintenance via regulating COUP-TFII. *eLife* **2016**, *5*, e21032. [CrossRef]
35. Sato, A.; Iwama, A.; Takakura, N.; Nishio, H.; Yancopoulos, G.D.; Suda, T. Characterization of TEK receptor tyrosine kinase and its ligands, Angiopoietins, in human hematopoietic progenitor cells. *Int. Immunol.* **1998**, *10*, 1217–1227. [CrossRef]
36. Yano, M.; Iwama, A.; Nishio, H.; Suda, J.; Takada, G.; Suda, T. Expression and function of murine receptor tyrosine kinases, TIE and TEK, in hematopoietic stem cells. *Blood* **1997**, *89*, 4317–4326. [CrossRef]

37. Joe, A.W.; Yi, L.; Natarajan, A.; Le Grand, F.; So, L.; Wang, J.; Rudnicki, M.A.; Rossi, F.M. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat. Cell Biol.* **2010**, *12*, 153–163. [CrossRef]
38. Uezumi, A.; Fukada, S.; Yamamoto, N.; Takeda, S.; Tsuchida, K. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nat. Cell Biol.* **2010**, *12*, 143–152. [CrossRef]
39. Dey, D.; Bagarova, J.; Hatsell, S.J.; Armstrong, K.A.; Huang, L.; Ermann, J.; Vonner, A.J.; Shen, Y.; Mohedas, A.H.; Lee, A.; et al. Two tissue-resident progenitor lineages drive distinct phenotypes of heterotopic ossification. *Sci. Transl. Med.* **2016**, *8*, 366ra163. [CrossRef]
40. Lees-Shepard, J.B.; Yamamoto, M.; Biswas, A.A.; Stoessel, S.J.; Nicholas, S.E.; Cogswell, C.A.; Devarakonda, P.M.; Schneider, M.J., Jr.; Cummins, S.M.; Legendre, N.P.; et al. Activin-dependent signaling in fibro/adipogenic progenitors causes fibrodysplasia ossificans progressiva. *Nat. Commun.* **2018**, *9*, 471. [CrossRef]
41. Agarwal, S.; Loder, S.; Brownley, C.; Cholok, D.; Mangiavini, L.; Li, J.; Breuler, C.; Sung, H.H.; Li, S.; Ranganathan, K.; et al. Inhibition of Hif1alpha prevents both trauma-induced and genetic heterotopic ossification. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E338–E347. [CrossRef] [PubMed]
42. Agarwal, S.; Loder, S.; Cholok, D.; Li, J.; Breuler, C.; Drake, J.; Brownley, C.; Peterson, J.; Li, S.; Levi, B. Surgical Excision of Heterotopic Ossification Leads to Re-Emergence of Mesenchymal Stem Cell Populations Responsible for Recurrence. *Stem Cells Transl. Med.* **2017**, *6*, 799–806. [CrossRef] [PubMed]
43. Agarwal, S.; Loder, S.J.; Cholok, D.; Peterson, J.; Li, J.; Breuler, C.; Brownley, R.C.; Sung, H.H.; Chung, M.T.; Kamiya, N.; et al. Scleraxis-Lineage Cells Contribute to Ectopic Bone Formation in Muscle and Tendon. *Stem Cells* **2017**, *35*, 705–710. [CrossRef]
44. Eisner, C.; Cummings, M.; Johnston, G.; Tung, L.W.; Groppa, E.; Chang, C.; Rossi, F.M. Murine Tissue-Resident PDGFRα+ Fibro-Adipogenic Progenitors Spontaneously Acquire Osteogenic Phenotype in an Altered Inflammatory Environment. *J. Bone Miner. Res.* **2020**, *35*, 1525–1534. [CrossRef]
45. Yin, H.; Price, F.; Rudnicki, M.A. Satellite cells and the muscle stem cell niche. *Physiol. Rev.* **2013**, *93*, 23–67. [CrossRef] [PubMed]
46. Charge, S.B.; Rudnicki, M.A. Cellular and molecular regulation of muscle regeneration. *Physiol. Rev.* **2004**, *84*, 209–238. [CrossRef] [PubMed]
47. Katagiri, T.; Yamaguchi, A.; Komaki, M.; Abe, E.; Takahashi, N.; Ikeda, T.; Rosen, V.; Wozney, J.M.; Fujisawa-Sehara, A.; Suda, T. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. *J. Cell Biol.* **1994**, *127*, 1755–1766. [CrossRef]
48. Hashimoto, N.; Kiyono, T.; Wada, M.R.; Umeda, R.; Goto, Y.; Nonaka, I.; Shimizu, S.; Yasumoto, S.; Inagawa-Ogashiwa, M. Osteogenic properties of human myogenic progenitor cells. *Mech. Dev.* **2008**, *125*, 257–269. [CrossRef]
49. Kan, L.; Liu, Y.; McGuire, T.L.; Berger, D.M.; Awatramani, R.B.; Dymecki, S.M.; Kessler, J.A. Dysregulation of local stem/progenitor cells as a common cellular mechanism for heterotopic ossification. *Stem Cells* **2009**, *27*, 150–156. [CrossRef]
50. Matthews, B.G.; Torreggiani, E.; Roeder, E.; Matic, I.; Grcevic, D.; Kalajzic, I. Osteogenic potential of alpha smooth muscle actin expressing muscle resident progenitor cells. *Bone* **2016**, *84*, 69–77. [CrossRef]
51. Shimono, K.; Tung, W.E.; Macolino, C.; Chi, A.H.; Didizian, J.H.; Mundy, C.; Chandraratna, R.A.; Mishina, Y.; Enomoto-Iwamoto, M.; Pacifici, M.; et al. Potent inhibition of heterotopic ossification by nuclear retinoic acid receptor-gamma agonists. *Nat. Med.* **2011**, *17*, 454–460. [CrossRef]
52. Winbanks, C.E.; Chen, J.L.; Qian, H.; Liu, Y.; Bernardo, B.C.; Beyer, C.; Watt, K.I.; Thomson, R.E.; Connor, T.; Turner, B.J.; et al. The bone morphogenetic protein axis is a positive regulator of skeletal muscle mass. *J. Cell Biol.* **2013**, *203*, 345–357. [CrossRef]
53. Liu, R.; Ginn, S.L.; Lek, M.; North, K.N.; Alexander, I.E.; Little, D.G.; Schindeler, A. Myoblast sensitivity and fibroblast insensitivity to osteogenic conversion by BMP-2 correlates with the expression of Bmpr-1a. *BMC Musculoskelet. Disord.* **2009**, *10*, 51. [CrossRef] [PubMed]
54. Wright, V.; Peng, H.; Usas, A.; Young, B.; Gearhart, B.; Cummins, J.; Huard, J. BMP4-expressing muscle-derived stem cells differentiate into osteogenic lineage and improve bone healing in immunocompetent mice. *Mol. Ther.* **2002**, *6*, 169–178. [CrossRef] [PubMed]
55. Wu, X.; Rathbone, C.R. Satellite cell functional alterations following cutaneous burn in rats include an increase in their osteogenic potential. *J. Surg. Res.* **2013**, *184*, e9–e16. [CrossRef]
56. Pagani, C.A.; Huber, A.K.; Hwang, C.; Marini, S.; Padmanabhan, K.; Livingston, N.; Nunez, J.; Sun, Y.; Edwards, N.; Cheng, Y.H.; et al. Novel Lineage-Tracing System to Identify Site-Specific Ectopic Bone Precursor Cells. *Stem Cell Rep.* **2021**, *16*, 626–640. [CrossRef]
57. Pineault, K.M.; Song, J.Y.; Kozloff, K.M.; Lucas, D.; Wellik, D.M. Hox11 expressing regional skeletal stem cells are progenitors for osteoblasts, chondrocytes and adipocytes throughout life. *Nat. Commun.* **2019**, *10*, 3168. [CrossRef]
58. Swinehart, I.T.; Schlientz, A.J.; Quintanilla, C.A.; Mortlock, D.P.; Wellik, D.M. Hox11 genes are required for regional patterning and integration of muscle, tendon and bone. *Development* **2013**, *140*, 4574–4582. [CrossRef] [PubMed]
59. Rux, D.R.; Song, J.Y.; Swinehart, I.T.; Pineault, K.M.; Schlientz, A.J.; Trulik, K.G.; Goldstein, S.A.; Kozloff, K.M.; Lucas, D.; Wellik, D.M. Regionally Restricted Hox Function in Adult Bone Marrow Multipotent Mesenchymal Stem/Stromal Cells. *Dev. Cell* **2016**, *39*, 653–666. [CrossRef]
60. Suda, R.K.; Billings, P.C.; Egan, K.P.; Kim, J.H.; McCarrick-Walmsley, R.; Glaser, D.L.; Porter, D.L.; Shore, E.M.; Pignolo, R.J. Circulating osteogenic precursor cells in heterotopic bone formation. *Stem Cells* **2009**, *27*, 2209–2219. [CrossRef]

61. Meyers, C.; Lisiecki, J.; Miller, S.; Levin, A.; Fayad, L.; Ding, C.; Sono, T.; McCarthy, E.; Levi, B.; James, A.W. Heterotopic Ossification: A Comprehensive Review. *JBMR Plus* **2019**, *3*, e10172. [CrossRef] [PubMed]
62. Grcevic, D.; Pejda, S.; Matthews, B.G.; Repic, D.; Wang, L.; Li, H.; Kronenberg, M.S.; Jiang, X.; Maye, P.; Adams, D.J.; et al. In vivo fate mapping identifies mesenchymal progenitor cells. *Stem Cells* **2012**, *30*, 187–196. [CrossRef] [PubMed]
63. Kan, L.; Peng, C.Y.; McGuire, T.L.; Kessler, J.A. Glast-expressing progenitor cells contribute to heterotopic ossification. *Bone* **2013**, *53*, 194–203. [CrossRef] [PubMed]
64. Slezak, M.; Goritz, C.; Niemiec, A.; Frisen, J.; Chambon, P.; Metzger, D.; Pfrieger, F.W. Transgenic mice for conditional gene manipulation in astroglial cells. *Glia* **2007**, *55*, 1565–1576. [CrossRef]
65. Howell, K.; Chien, C.; Bell, R.; Laudier, D.; Tufa, S.F.; Keene, D.R.; Andarawis-Puri, N.; Huang, A.H. Novel Model of Tendon Regeneration Reveals Distinct Cell Mechanisms Underlying Regenerative and Fibrotic Tendon Healing. *Sci. Rep.* **2017**, *7*, 45238. [CrossRef]
66. Kan, L.; Lounev, V.Y.; Pignolo, R.J.; Duan, L.; Liu, Y.; Stock, S.R.; McGuire, T.L.; Lu, B.; Gerard, N.P.; Shore, E.M.; et al. Substance P signaling mediates BMP-dependent heterotopic ossification. *J. Cell Biochem.* **2011**, *112*, 2759–2772. [CrossRef]
67. Lazard, Z.W.; Olmsted-Davis, E.A.; Salisbury, E.A.; Gugala, Z.; Sonnet, C.; Davis, E.L.; Beal, E., 2nd; Ubogu, E.E.; Davis, A.R. Osteoblasts Have a Neural Origin in Heterotopic Ossification. *Clin. Orthop. Relat. Res.* **2015**, *473*, 2790–2806. [CrossRef]
68. Salisbury, E.; Rodenberg, E.; Sonnet, C.; Hipp, J.; Gannon, F.H.; Vadakkan, T.J.; Dickinson, M.E.; Olmsted-Davis, E.A.; Davis, A.R. Sensory nerve induced inflammation contributes to heterotopic ossification. *J. Cell. Biochem.* **2011**, *112*, 2748–2758. [CrossRef]
69. Salisbury, E.; Sonnet, C.; Heggeness, M.; Davis, A.R.; Olmsted-Davis, E. Heterotopic ossification has some nerve. *Crit. Rev. Eukaryot. Gene Expr.* **2010**, *20*, 313–324. [CrossRef]
70. Olmsted-Davis, E.; Gannon, F.H.; Ozen, M.; Ittmann, M.M.; Gugala, Z.; Hipp, J.A.; Moran, K.M.; Fouletier-Dilling, C.M.; Schumara-Martin, S.; Lindsey, R.W.; et al. Hypoxic adipocytes pattern early heterotopic bone formation. *Am. J. Pathol.* **2007**, *170*, 620–632. [CrossRef]
71. Salisbury, E.A.; Dickerson, A.R.; Davis, T.A.; Forsberg, J.A.; Davis, A.R.; Olmsted-Davis, E.A. Characterization of Brown Adipose-Like Tissue in Trauma-Induced Heterotopic Ossification in Humans. *Am. J. Pathol.* **2017**, *187*, 2071–2079. [CrossRef] [PubMed]
72. Urist, M.R. Bone: Formation by autoinduction. *Science* **1965**, *150*, 893–899. [CrossRef] [PubMed]
73. Wang, R.N.; Green, J.; Wang, Z.; Deng, Y.; Qiao, M.; Peabody, M.; Zhang, Q.; Ye, J.; Yan, Z.; Denduluri, S.; et al. Bone Morphogenetic Protein (BMP) signaling in development and human diseases. *Genes Dis.* **2014**, *1*, 87–105. [CrossRef] [PubMed]
74. Asharani, P.V.; Keupp, K.; Semler, O.; Wang, W.; Li, Y.; Thiele, H.; Yigit, G.; Pohl, E.; Becker, J.; Frommolt, P.; et al. Attenuated BMP1 function compromises osteogenesis, leading to bone fragility in humans and zebrafish. *Am. J. Hum. Genet.* **2012**, *90*, 661–674. [CrossRef]
75. Chen, D.; Zhao, M.; Mundy, G.R. Bone morphogenetic proteins. *Growth Factors* **2004**, *22*, 233–241. [CrossRef]
76. Ma, L.; Lu, M.F.; Schwartz, R.J.; Martin, J.F. Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. *Development* **2005**, *132*, 5601–5611. [CrossRef]
77. Rahman, M.S.; Akhtar, N.; Jamil, H.M.; Banik, R.S.; Asaduzzaman, S.M. TGF-beta/BMP signaling and other molecular events: Regulation of osteoblastogenesis and bone formation. *Bone Res.* **2015**, *3*, 15005. [CrossRef]
78. Zhang, H.; Bradley, A. Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development* **1996**, *122*, 2977–2986. [CrossRef]
79. Faucheux, C.; Ulysse, F.; Bareille, R.; Reddi, A.H.; Amedee, J. Opposing actions of BMP3 and TGF beta 1 in human bone marrow stromal cell growth and differentiation. *Biochem. Biophys. Res. Commun.* **1997**, *241*, 787–793. [CrossRef]
80. Cole, A.E.; Murray, S.S.; Xiao, J. Bone Morphogenetic Protein 4 Signalling in Neural Stem and Progenitor Cells during Development and after Injury. *Stem Cells Int.* **2016**, *2016*, 9260592. [CrossRef]
81. Lee, T.J.; Jang, J.; Kang, S.; Jin, M.; Shin, H.; Kim, D.W.; Kim, B.S. Enhancement of osteogenic and chondrogenic differentiation of human embryonic stem cells by mesodermal lineage induction with BMP-4 and FGF2 treatment. *Biochem. Biophys. Res. Commun.* **2013**, *430*, 793–797. [CrossRef] [PubMed]
82. Modica, S.; Wolfrum, C. The dual role of BMP4 in adipogenesis and metabolism. *Adipocyte* **2017**, *6*, 141–146. [CrossRef] [PubMed]
83. Watanabe, Y.; Le Douarin, N.M. A role for BMP-4 in the development of subcutaneous cartilage. *Mech. Dev.* **1996**, *57*, 69–78. [CrossRef]
84. Bobinac, D.; Maric, I.; Zoricic, S.; Spanjol, J.; Dordevic, G.; Mustac, E.; Fuckar, Z. Expression of bone morphogenetic proteins in human metastatic prostate and breast cancer. *Croat. Med. J.* **2005**, *46*, 389–396. [PubMed]
85. Guenther, C.A.; Wang, Z.; Li, E.; Tran, M.C.; Logan, C.Y.; Nusse, R.; Pantalena-Filho, L.; Yang, G.P.; Kingsley, D.M. A distinct regulatory region of the Bmp5 locus activates gene expression following adult bone fracture or soft tissue injury. *Bone* **2015**, *77*, 31–41. [CrossRef]
86. Wordinger, R.J.; Agarwal, R.; Talati, M.; Fuller, J.; Lambert, W.; Clark, A.F. Expression of bone morphogenetic proteins (BMP), BMP receptors, and BMP associated proteins in human trabecular meshwork and optic nerve head cells and tissues. *Mol. Vis.* **2002**, *8*, 241–250.
87. Camaschella, C. BMP6 orchestrates iron metabolism. *Nat. Genet.* **2009**, *41*, 386–388. [CrossRef]
88. Gitelman, S.E.; Kobrin, M.S.; Ye, J.Q.; Lopez, A.R.; Lee, A.; Derynck, R. Recombinant Vgr-1/BMP-6-expressing tumors induce fibrosis and endochondral bone formation in vivo. *J. Cell Biol.* **1994**, *126*, 1595–1609. [CrossRef]

89. Hahn, G.V.; Cohen, R.B.; Wozney, J.M.; Levitz, C.L.; Shore, E.M.; Zasloff, M.A.; Kaplan, F.S. A bone morphogenetic protein subfamily: Chromosomal localization of human genes for BMP5, BMP6, and BMP7. *Genomics* **1992**, *14*, 759–762. [CrossRef]
90. Dudley, A.T.; Lyons, K.M.; Robertson, E.J. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev.* **1995**, *9*, 2795–2807. [CrossRef]
91. Itoh, F.; Asao, H.; Sugamura, K.; Heldin, C.H.; ten Dijke, P.; Itoh, S. Promoting bone morphogenetic protein signaling through negative regulation of inhibitory Smads. *EMBO J.* **2001**, *20*, 4132–4142. [CrossRef]
92. Tseng, Y.H.; Kokkotou, E.; Schulz, T.J.; Huang, T.L.; Winnay, J.N.; Taniguchi, C.M.; Tran, T.T.; Suzuki, R.; Espinoza, D.O.; Yamamoto, Y.; et al. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* **2008**, *454*, 1000–1004. [CrossRef] [PubMed]
93. DiLeone, R.J.; King, J.A.; Storm, E.E.; Copeland, N.G.; Jenkins, N.A.; Kingsley, D.M. The Bmp8 gene is expressed in developing skeletal tissue and maps near the Achondroplasia locus on mouse chromosome 4. *Genomics* **1997**, *40*, 196–198. [CrossRef] [PubMed]
94. Ying, Y.; Liu, X.M.; Marble, A.; Lawson, K.A.; Zhao, G.Q. Requirement of Bmp8b for the generation of primordial germ cells in the mouse. *Mol. Endocrinol.* **2000**, *14*, 1053–1063. [CrossRef] [PubMed]
95. Zhao, G.Q.; Deng, K.; Labosky, P.A.; Liaw, L.; Hogan, B.L. The gene encoding bone morphogenetic protein 8B is required for the initiation and maintenance of spermatogenesis in the mouse. *Genes Dev.* **1996**, *10*, 1657–1669. [CrossRef] [PubMed]
96. Zhao, G.Q.; Liaw, L.; Hogan, B.L. Bone morphogenetic protein 8A plays a role in the maintenance of spermatogenesis and the integrity of the epididymis. *Development* **1998**, *125*, 1103–1112. [CrossRef] [PubMed]
97. Kang, Q.; Sun, M.H.; Cheng, H.; Peng, Y.; Montag, A.G.; Deyrup, A.T.; Jiang, W.; Luu, H.H.; Luo, J.; Szatkowski, J.P.; et al. Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. *Gene Ther.* **2004**, *11*, 1312–1320. [CrossRef]
98. Levet, S.; Ciais, D.; Merdzhanova, G.; Mallet, C.; Zimmers, T.A.; Lee, S.J.; Navarro, F.P.; Texier, I.; Feige, J.J.; Bailly, S.; et al. Bone morphogenetic protein 9 (BMP9) controls lymphatic vessel maturation and valve formation. *Blood* **2013**, *122*, 598–607. [CrossRef]
99. Majumdar, M.K.; Wang, E.; Morris, E.A. BMP-2 and BMP-9 promotes chondrogenic differentiation of human multipotential mesenchymal cells and overcomes the inhibitory effect of IL-1. *J. Cell Physiol.* **2001**, *189*, 275–284. [CrossRef]
100. Chen, H.; Shi, S.; Acosta, L.; Li, W.; Lu, J.; Bao, S.; Chen, Z.; Yang, Z.; Schneider, M.D.; Chien, K.R.; et al. BMP10 is essential for maintaining cardiac growth during murine cardiogenesis. *Development* **2004**, *131*, 2219–2231. [CrossRef]
101. Mitrofan, C.G.; Appleby, S.L.; Nash, G.B.; Mallat, Z.; Chilvers, E.R.; Upton, P.D.; Morrell, N.W. Bone morphogenetic protein 9 (BMP9) and BMP10 enhance tumor necrosis factor-alpha-induced monocyte recruitment to the vascular endothelium mainly via activin receptor-like kinase 2. *J. Biol. Chem.* **2017**, *292*, 13714–13726. [CrossRef]
102. Neuhaus, H.; Rosen, V.; Thies, R.S. Heart specific expression of mouse BMP-10 a novel member of the TGF-beta superfamily. *Mech. Dev.* **1999**, *80*, 181–184. [CrossRef]
103. Li, Z.; Zeng, F.; Mitchell, A.D.; Kim, Y.S.; Wu, Z.; Yang, J. Transgenic overexpression of bone morphogenetic protein 11 propeptide in skeleton enhances bone formation. *Biochem. Biophys. Res. Commun.* **2011**, *416*, 289–292. [CrossRef]
104. Zhang, Y.; Wei, Y.; Liu, D.; Liu, F.; Li, X.; Pan, L.; Pang, Y.; Chen, D. Role of growth differentiation factor 11 in development, physiology and disease. *Oncotarget* **2017**, *8*, 81604–81616. [CrossRef] [PubMed]
105. Berasi, S.P.; Varadarajan, U.; Archambault, J.; Cain, M.; Souza, T.A.; Abouzeid, A.; Li, J.; Brown, C.T.; Dorner, A.J.; Seeherman, H.J.; et al. Divergent activities of osteogenic BMP2, and tenogenic BMP12 and BMP13 independent of receptor binding affinities. *Growth Factors* **2011**, *29*, 128–139. [CrossRef] [PubMed]
106. Settle, S.H., Jr.; Rountree, R.B.; Sinha, A.; Thacker, A.; Higgins, K.; Kingsley, D.M. Multiple joint and skeletal patterning defects caused by single and double mutations in the mouse Gdf6 and Gdf5 genes. *Dev. Biol.* **2003**, *254*, 116–130. [CrossRef]
107. Chhabra, A.; Zijerdi, D.; Zhang, J.; Kline, A.; Balian, G.; Hurwitz, S. BMP-14 deficiency inhibits long bone fracture healing: A biochemical, histologic, and radiographic assessment. *J. Orthop. Trauma* **2005**, *19*, 629–634. [CrossRef] [PubMed]
108. Coleman, C.M.; Scheremeta, B.H.; Boyce, A.T.; Mauck, R.L.; Tuan, R.S. Delayed fracture healing in growth differentiation factor 5-deficient mice: A pilot study. *Clin. Orthop. Relat. Res.* **2011**, *469*, 2915–2924. [CrossRef]
109. Di Pasquale, E.; Beck-Peccoz, P.; Persani, L. Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. *Am. J. Hum. Genet.* **2004**, *75*, 106–111. [CrossRef]
110. Yan, C.; Wang, P.; DeMayo, J.; DeMayo, F.J.; Elvin, J.A.; Carino, C.; Prasad, S.V.; Skinner, S.S.; Dunbar, B.S.; Dube, J.L.; et al. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol. Endocrinol.* **2001**, *15*, 854–866. [CrossRef]
111. Wang, M.; Jin, H.; Tang, D.; Huang, S.; Zuscik, M.J.; Chen, D. Smad1 plays an essential role in bone development and postnatal bone formation. *Osteoarthr. Cartil.* **2011**, *19*, 751–762. [CrossRef] [PubMed]
112. Iwasaki, S.; Iguchi, M.; Watanabe, K.; Hoshino, R.; Tsujimoto, M.; Kohno, M. Specific activation of the p38 mitogen-activated protein kinase signaling pathway and induction of neurite outgrowth in PC12 cells by bone morphogenetic protein-2. *J. Biol. Chem.* **1999**, *274*, 26503–26510. [CrossRef] [PubMed]
113. Lou, J.; Tu, Y.; Li, S.; Manske, P.R. Involvement of ERK in BMP-2 induced osteoblastic differentiation of mesenchymal progenitor cell line C3H10T1/2. *Biochem. Biophys. Res. Commun.* **2000**, *268*, 757–762. [CrossRef] [PubMed]

114. Nakamura, K.; Shirai, T.; Morishita, S.; Uchida, S.; Saeki-Miura, K.; Makishima, F. p38 mitogen-activated protein kinase functionally contributes to chondrogenesis induced by growth/differentiation factor-5 in ATDC5 cells. *Exp. Cell Res.* **1999**, *250*, 351–363. [CrossRef] [PubMed]
115. Yamaguchi, K.; Shirakabe, K.; Shibuya, H.; Irie, K.; Oishi, I.; Ueno, N.; Taniguchi, T.; Nishida, E.; Matsumoto, K. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. *Science* **1995**, *270*, 2008–2011. [CrossRef] [PubMed]
116. Bloise, E.; Ciarmela, P.; Dela Cruz, C.; Luisi, S.; Petraglia, F.; Reis, F.M. Activin A in Mammalian Physiology. *Physiol. Rev.* **2019**, *99*, 739–780. [CrossRef] [PubMed]
117. de Caestecker, M.P.; Parks, W.T.; Frank, C.J.; Castagnino, P.; Bottaro, D.P.; Roberts, A.B.; Lechleider, R.J. Smad2 transduces common signals from receptor serine-threonine and tyrosine kinases. *Genes Dev.* **1998**, *12*, 1587–1592. [CrossRef]
118. Gingery, A.; Bradley, E.W.; Pederson, L.; Ruan, M.; Horwood, N.J.; Oursler, M.J. TGF-beta coordinately activates TAK1/MEK/AKT/NFkB and SMAD pathways to promote osteoclast survival. *Exp. Cell Res.* **2008**, *314*, 2725–2738. [CrossRef]
119. Hoffmann, A.; Preobrazhenska, O.; Wodarczyk, C.; Medler, Y.; Winkel, A.; Shahab, S.; Huylebroeck, D.; Gross, G.; Verschueren, K. Transforming growth factor-beta-activated kinase-1 (TAK1), a MAP3K, interacts with Smad proteins and interferes with osteogenesis in murine mesenchymal progenitors. *J. Biol. Chem.* **2005**, *280*, 27271–27283. [CrossRef]
120. Nickel, J.; Mueller, T.D. Specification of BMP Signaling. *Cells* **2019**, *8*, 1579. [CrossRef]
121. Evans, K.N.; Potter, B.K.; Brown, T.S.; Davis, T.A.; Elster, E.A.; Forsberg, J.A. Osteogenic gene expression correlates with development of heterotopic ossification in war wounds. *Clin. Orthop. Relat. Res.* **2014**, *472*, 396–404. [CrossRef] [PubMed]
122. Ju, C.; Lv, Z.; Zhang, C.; Jiao, Y. Regulatory effect of miR-421 on humeral fracture and heterotopic ossification in elderly patients. *Exp. Ther. Med.* **2019**, *17*, 1903–1911. [CrossRef]
123. Hannallah, D.; Peng, H.; Young, B.; Usas, A.; Gearhart, B.; Huard, J. Retroviral delivery of Noggin inhibits the formation of heterotopic ossification induced by BMP-4, demineralized bone matrix, and trauma in an animal model. *J. Bone Jt. Surg. Am.* **2004**, *86*, 80–91. [CrossRef] [PubMed]
124. Yu, P.B.; Deng, D.Y.; Lai, C.S.; Hong, C.C.; Cuny, G.D.; Bouxsein, M.L.; Hong, D.W.; McManus, P.M.; Katagiri, T.; Sachidanandan, C.; et al. BMP type I receptor inhibition reduces heterotopic [corrected] ossification. *Nat. Med.* **2008**, *14*, 1363–1369. [CrossRef] [PubMed]
125. Kan, L.; Hu, M.; Gomes, W.A.; Kessler, J.A. Transgenic mice overexpressing BMP4 develop a fibrodysplasia ossificans progressiva (FOP)-like phenotype. *Am. J. Pathol.* **2004**, *165*, 1107–1115. [CrossRef]
126. Scott, M.A.; Levi, B.; Askarinam, A.; Nguyen, A.; Rackohn, T.; Ting, K.; Soo, C.; James, A.W. Brief review of models of ectopic bone formation. *Stem Cells Dev.* **2012**, *21*, 655–667. [CrossRef]
127. Ranganathan, K.; Loder, S.; Agarwal, S.; Wong, V.W.; Forsberg, J.; Davis, T.A.; Wang, S.; James, A.W.; Levi, B. Heterotopic Ossification: Basic-Science Principles and Clinical Correlates. *J. Bone Jt. Surg. Am.* **2015**, *97*, 1101–1111. [CrossRef]
128. Chen, J.; Long, F. mTOR signaling in skeletal development and disease. *Bone Res.* **2018**, *6*, 1. [CrossRef]
129. Phornphutkul, C.; Wu, K.Y.; Auyeung, V.; Chen, Q.; Gruppuso, P.A. mTOR signaling contributes to chondrocyte differentiation. *Dev. Dyn* **2008**, *237*, 702–712. [CrossRef]
130. Hino, K.; Horigome, K.; Nishio, M.; Komura, S.; Nagata, S.; Zhao, C.; Jin, Y.; Kawakami, K.; Yamada, Y.; Ohta, A.; et al. Activin-A enhances mTOR signaling to promote aberrant chondrogenesis in fibrodysplasia ossificans progressiva. *J. Clin. Investig.* **2017**, *127*, 3339–3352. [CrossRef]
131. Qureshi, A.T.; Dey, D.; Sanders, E.M.; Seavey, J.G.; Tomasino, A.M.; Moss, K.; Wheatley, B.; Cholok, D.; Loder, S.; Li, J.; et al. Inhibition of Mammalian Target of Rapamycin Signaling with Rapamycin Prevents Trauma-Induced Heterotopic Ossification. *Am. J. Pathol.* **2017**, *187*, 2536–2545. [CrossRef] [PubMed]
132. Jiang, H.; Chen, Y.; Chen, G.; Tian, X.; Tang, J.; Luo, L.; Huang, M.; Yan, B.; Ao, X.; Zhou, W.; et al. Leptin accelerates the pathogenesis of heterotopic ossification in rat tendon tissues via mTORC1 signaling. *J. Cell. Physiol.* **2018**, *233*, 1017–1028. [CrossRef] [PubMed]
133. Meng, D.; Frank, A.R.; Jewell, J.L. mTOR signaling in stem and progenitor cells. *Development* **2018**, *145*, dev152595. [CrossRef] [PubMed]
134. Karner, C.M.; Lee, S.Y.; Long, F. Bmp Induces Osteoblast Differentiation through both Smad4 and mTORC1 Signaling. *Mol. Cell. Biol.* **2017**, *37*, e00253-16. [CrossRef]
135. Chen, J.; Holguin, N.; Shi, Y.; Silva, M.J.; Long, F. mTORC2 signaling promotes skeletal growth and bone formation in mice. *J. Bone Miner. Res.* **2015**, *30*, 369–378. [CrossRef]
136. Sun, W.; Shi, Y.; Lee, W.C.; Lee, S.Y.; Long, F. Rictor is required for optimal bone accrual in response to anti-sclerostin therapy in the mouse. *Bone* **2016**, *85*, 1–8. [CrossRef]
137. Semenza, G.L. Hypoxia-inducible factor 1: Oxygen homeostasis and disease pathophysiology. *Trends Mol. Med.* **2001**, *7*, 345–350. [CrossRef]
138. Wang, Y.; Wan, C.; Deng, L.; Liu, X.; Cao, X.; Gilbert, S.R.; Bouxsein, M.L.; Faugere, M.C.; Guldberg, R.E.; Gerstenfeld, L.C.; et al. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. *J. Clin. Investig.* **2007**, *117*, 1616–1626. [CrossRef]

139. Galdones, E.; Hales, B.F. Retinoic acid receptor gamma-induced misregulation of chondrogenesis in the murine limb bud in vitro. *Toxicol. Sci.* **2008**, *106*, 223–232. [CrossRef]
140. Romand, R.; Hashino, E.; Dolle, P.; Vonesch, J.L.; Chambon, P.; Ghyselinck, N.B. The retinoic acid receptors RARalpha and RARgamma are required for inner ear development. *Mech. Dev.* **2002**, *119*, 213–223. [CrossRef]
141. Weston, A.D.; Chandraratna, R.A.; Torchia, J.; Underhill, T.M. Requirement for RAR-mediated gene repression in skeletal progenitor differentiation. *J. Cell Biol.* **2002**, *158*, 39–51. [CrossRef] [PubMed]
142. Weston, A.D.; Rosen, V.; Chandraratna, R.A.; Underhill, T.M. Regulation of skeletal progenitor differentiation by the BMP and retinoid signaling pathways. *J. Cell Biol.* **2000**, *148*, 679–690. [CrossRef] [PubMed]
143. Cash, D.E.; Bock, C.B.; Schughart, K.; Linney, E.; Underhill, T.M. Retinoic acid receptor alpha function in vertebrate limb skeletogenesis: A modulator of chondrogenesis. *J. Cell Biol.* **1997**, *136*, 445–457. [CrossRef] [PubMed]
144. Kan, C.; Chen, L.; Hu, Y.; Ding, N.; Lu, H.; Li, Y.; Kessler, J.A.; Kan, L. Conserved signaling pathways underlying heterotopic ossification. *Bone* **2018**, *109*, 43–48. [CrossRef]
145. Liu, X.; Qin, J.; Luo, Q.; Bi, Y.; Zhu, G.; Jiang, W.; Kim, S.H.; Li, M.; Su, Y.; Nan, G.; et al. Cross-talk between EGF and BMP9 signalling pathways regulates the osteogenic differentiation of mesenchymal stem cells. *J. Cell. Mol. Med.* **2013**, *17*, 1160–1172. [CrossRef] [PubMed]
146. Luo, K. Signaling Cross Talk between TGF-beta/Smad and Other Signaling Pathways. *Cold Spring Harb. Perspect. Biol.* **2017**, *9*, a022137. [CrossRef]
147. Nakashima, A.; Katagiri, T.; Tamura, M. Cross-talk between Wnt and bone morphogenetic protein 2 (BMP-2) signaling in differentiation pathway of C2C12 myoblasts. *J. Biol. Chem.* **2005**, *280*, 37660–37668. [CrossRef]
148. Zhang, T.; Wen, F.; Wu, Y.; Goh, G.S.; Ge, Z.; Tan, L.P.; Hui, J.H.; Yang, Z. Cross-talk between TGF-beta/SMAD and integrin signaling pathways in regulating hypertrophy of mesenchymal stem cell chondrogenesis under deferral dynamic compression. *Biomaterials* **2015**, *38*, 72–85. [CrossRef]
149. Pavlou, G.; Kyrkos, M.; Tsialogiannis, E.; Korres, N.; Tsiridis, E. Pharmacological treatment of heterotopic ossification following hip surgery: An update. *Expert Opin. Pharm.* **2012**, *13*, 619–622. [CrossRef]
150. Joice, M.; Vasileiadis, G.I.; Amanatullah, D.F. Non-steroidal anti-inflammatory drugs for heterotopic ossification prophylaxis after total hip arthroplasty: A systematic review and meta-analysis. *Bone Jt. J.* **2018**, *100-B*, 915–922. [CrossRef]
151. Kan, S.L.; Yang, B.; Ning, G.Z.; Chen, L.X.; Li, Y.L.; Gao, S.J.; Chen, X.Y.; Sun, J.C.; Feng, S.Q. Nonsteroidal Anti-inflammatory Drugs as Prophylaxis for Heterotopic Ossification after Total Hip Arthroplasty: A Systematic Review and Meta-Analysis. *Medicine* **2015**, *94*, e828. [CrossRef] [PubMed]
152. Migliorini, F.; Trivellas, A.; Eschweiler, J.; Driessen, A.; Tingart, M.; Maffulli, N. NSAIDs for Prophylaxis for Heterotopic Ossification After Total Hip Arthroplasty: A Bayesian Network Meta-analysis. *Calcif. Tissue Int.* **2021**, *108*, 196–206. [CrossRef] [PubMed]
153. Hu, Z.H.; Chen, W.; Sun, J.N.; Zhang, Y.; Zhang, Y.; Chen, X.Y.; Feng, S. Radiotherapy for the prophylaxis of heterotopic ossification after total hip arthroplasty: A systematic review and meta-analysis of randomized controlled trails. *Med. Dosim.* **2021**, *46*, 65–73. [CrossRef] [PubMed]
154. Popovic, M.; Agarwal, A.; Zhang, L.; Yip, C.; Kreder, H.J.; Nousiainen, M.T.; Jenkinson, R.; Tsao, M.; Lam, H.; Milakovic, M.; et al. Radiotherapy for the prophylaxis of heterotopic ossification: A systematic review and meta-analysis of published data. *Radiother. Oncol.* **2014**, *113*, 10–17. [CrossRef] [PubMed]
155. Sheybani, A.; TenNapel, M.J.; Lack, W.D.; Clerkin, P.; Hyer, D.E.; Sun, W.; Jacobson, G.M. Risk of radiation-induced malignancy with heterotopic ossification prophylaxis: A case-control analysis. *Int. J. Radiat. Oncol. Biol. Phys.* **2014**, *89*, 584–589. [CrossRef]
156. Balboni, T.A.; Gobezie, R.; Mamon, H.J. Heterotopic ossification: Pathophysiology, clinical features, and the role of radiotherapy for prophylaxis. *Int. J. Radiat. Oncol. Biol. Phys.* **2006**, *65*, 1289–1299. [CrossRef] [PubMed]
157. Hamid, N.; Ashraf, N.; Bosse, M.J.; Connor, P.M.; Kellam, J.F.; Sims, S.H.; Stull, D.E.; Jeray, K.J.; Hymes, R.A.; Lowe, T.J. Radiation therapy for heterotopic ossification prophylaxis acutely after elbow trauma: A prospective randomized study. *J. Bone Jt. Surg. Am.* **2010**, *92*, 2032–2038. [CrossRef]
158. Pavey, G.J.; Polfer, E.M.; Nappo, K.E.; Tintle, S.M.; Forsberg, J.A.; Potter, B.K. What Risk Factors Predict Recurrence of Heterotopic Ossification After Excision in Combat-related Amputations? *Clin. Orthop. Relat. Res.* **2015**, *473*, 2814–2824. [CrossRef]
159. Meiners, T.; Abel, R.; Bohm, V.; Gerner, H.J. Resection of heterotopic ossification of the hip in spinal cord injured patients. *Spinal Cord* **1997**, *35*, 443–445. [CrossRef]
160. Lee, E.K.; Namdari, S.; Hosalkar, H.S.; Keenan, M.A.; Baldwin, K.D. Clinical results of the excision of heterotopic bone around the elbow: A systematic review. *J. Shoulder Elb. Surg.* **2013**, *22*, 716–722. [CrossRef]
161. Thomas, B.J.; Amstutz, H.C. Results of the administration of diphosphonate for the prevention of heterotopic ossification after total hip arthroplasty. *J. Bone Jt. Surg. Am.* **1985**, *67*, 400–403. [CrossRef]
162. Shafer, D.M.; Bay, C.; Caruso, D.M.; Foster, K.N. The use of eidronate disodium in the prevention of heterotopic ossification in burn patients. *Burns* **2008**, *34*, 355–360. [CrossRef] [PubMed]
163. Shimono, K.; Morrison, T.N.; Tung, W.E.; Chandraratna, R.A.; Williams, J.A.; Iwamoto, M.; Pacifici, M. Inhibition of ectopic bone formation by a selective retinoic acid receptor alpha-agonist: A new therapy for heterotopic ossification? *J. Orthop. Res.* **2010**, *28*, 271–277. [CrossRef] [PubMed]

164. Koyama, E.; Golden, E.B.; Kirsch, T.; Adams, S.L.; Chandraratna, R.A.; Michaille, J.J.; Pacifici, M. Retinoid signaling is required for chondrocyte maturation and endochondral bone formation during limb skeletogenesis. *Dev. Biol.* **1999**, *208*, 375–391. [CrossRef] [PubMed]
165. Weston, A.D.; Hoffman, L.M.; Underhill, T.M. Revisiting the role of retinoid signaling in skeletal development. *Birth Defects Res. C Embryo Today* **2003**, *69*, 156–173. [CrossRef] [PubMed]
166. Chakkalakal, S.A.; Uchibe, K.; Convente, M.R.; Zhang, D.; Economides, A.N.; Kaplan, F.S.; Pacifici, M.; Iwamoto, M.; Shore, E.M. Palovarotene Inhibits Heterotopic Ossification and Maintains Limb Mobility and Growth in Mice with the Human ACVR1(R206H) Fibrodysplasia Ossificans Progressiva (FOP) Mutation. *J. Bone Miner. Res.* **2016**, *31*, 1666–1675. [CrossRef] [PubMed]
167. Lees-Shepard, J.B.; Nicholas, S.E.; Stoessel, S.J.; Devarakonda, P.M.; Schneider, M.J.; Yamamoto, M.; Goldhamer, D.J. Palovarotene reduces heterotopic ossification in juvenile FOP mice but exhibits pronounced skeletal toxicity. *eLife* **2018**, *7*, e40814. [CrossRef]
168. Wheatley, B.M.; Cilwa, K.E.; Dey, D.; Qureshi, A.T.; Seavey, J.G.; Tomasino, A.M.; Sanders, E.M.; Bova, W.; Boehm, C.A.; Iwamoto, M.; et al. Palovarotene inhibits connective tissue progenitor cell proliferation in a rat model of combat-related heterotopic ossification. *J. Orthop. Res.* **2018**, *36*, 1135–1144. [CrossRef]
169. Pavey, G.J.; Qureshi, A.T.; Tomasino, A.M.; Honnold, C.L.; Bishop, D.K.; Agarwal, S.; Loder, S.; Levi, B.; Pacifici, M.; Iwamoto, M.; et al. Targeted stimulation of retinoic acid receptor-gamma mitigates the formation of heterotopic ossification in an established blast-related traumatic injury model. *Bone* **2016**, *90*, 159–167. [CrossRef]
170. Lebrun, J.J.; Takabe, K.; Chen, Y.; Vale, W. Roles of pathway-specific and inhibitory Smads in activin receptor signaling. *Mol. Endocrinol.* **1999**, *13*, 15–23. [CrossRef]
171. Mueller, T.D.; Nickel, J. Promiscuity and specificity in BMP receptor activation. *FEBS Lett.* **2012**, *586*, 1846–1859. [CrossRef] [PubMed]
172. Massague, J.; Seoane, J.; Wotton, D. Smad transcription factors. *Genes Dev.* **2005**, *19*, 2783–2810. [CrossRef] [PubMed]
173. Yang, J.; Jiang, W. The Role of SMAD2/3 in Human Embryonic Stem Cells. *Front. Cell Dev. Biol.* **2020**, *8*, 653. [CrossRef] [PubMed]
174. Yang, J.; Wahdan-Alaswad, R.; Danielpour, D. Critical role of Smad2 in tumor suppression and transforming growth factor-beta-induced apoptosis of prostate epithelial cells. *Cancer Res.* **2009**, *69*, 2185–2190. [CrossRef]
175. Culbert, A.L.; Chakkalakal, S.A.; Theosmy, E.G.; Brennan, T.A.; Kaplan, F.S.; Shore, E.M. Alk2 regulates early chondrogenic fate in fibrodysplasia ossificans progressiva heterotopic endochondral ossification. *Stem Cells* **2014**, *32*, 1289–1300. [CrossRef]
176. van Dinther, M.; Visser, N.; de Gorter, D.J.; Doorn, J.; Goumans, M.J.; de Boer, J.; ten Dijke, P. ALK2 R206H mutation linked to fibrodysplasia ossificans progressiva confers constitutive activity to the BMP type I receptor and sensitizes mesenchymal cells to BMP-induced osteoblast differentiation and bone formation. *J. Bone Miner. Res.* **2010**, *25*, 1208–1215. [CrossRef]
177. Lyu, H.; Elkins, C.M.; Pierce, J.L.; Serezani, C.H.; Perrien, D.S. MyD88 Is Not Required for Muscle Injury-Induced Endochondral Heterotopic Ossification in a Mouse Model of Fibrodysplasia Ossificans Progressiva. *Biomedicines* **2021**, *9*, 630. [CrossRef]
178. Lin, H.; Ying, Y.; Wang, Y.Y.; Wang, G.; Jiang, S.S.; Huang, D.; Luo, L.; Chen, Y.G.; Gerstenfeld, L.C.; Luo, Z. AMPK downregulates ALK2 via increasing the interaction between Smurf1 and Smad6, leading to inhibition of osteogenic differentiation. *Biochim. Biophys. Acta Mol. Cell Res.* **2017**, *1864*, 2369–2377. [CrossRef]
179. Hatsell, S.J.; Idone, V.; Wolken, D.M.; Huang, L.; Kim, H.J.; Wang, L.; Wen, X.; Nannuru, K.C.; Jimenez, J.; Xie, L.; et al. ACVR1R206H receptor mutation causes fibrodysplasia ossificans progressiva by imparting responsiveness to activin A. *Sci. Transl. Med.* **2015**, *7*, 303ra137. [CrossRef]
180. Hino, K.; Ikeya, M.; Horigome, K.; Matsumoto, Y.; Ebise, H.; Nishio, M.; Sekiguchi, K.; Shibata, M.; Nagata, S.; Matsuda, S.; et al. Neofunction of ACVR1 in fibrodysplasia ossificans progressiva. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 15438–15443. [CrossRef]
181. Song, G.A.; Kim, H.J.; Woo, K.M.; Baek, J.H.; Kim, G.S.; Choi, J.Y.; Ryoo, H.M. Molecular consequences of the ACVR1(R206H) mutation of fibrodysplasia ossificans progressiva. *J. Biol. Chem.* **2010**, *285*, 22542–22553. [CrossRef]
182. Kaplan, F.S.; Le Merrer, M.; Glaser, D.L.; Pignolo, R.J.; Goldsby, R.E.; Kitterman, J.A.; Groppe, J.; Shore, E.M. Fibrodysplasia ossificans progressiva. *Best Pract. Res. Clin. Rheumatol.* **2008**, *22*, 191–205. [CrossRef] [PubMed]
183. Lin, H.; Shi, F.; Gao, J.; Hua, P. The role of Activin A in fibrodysplasia ossificans progressiva: A prominent mediator. *Biosci. Rep.* **2019**, *39*, BSR20190377. [CrossRef] [PubMed]
184. Ramirez, D.M.; Ramirez, M.R.; Reginato, A.M.; Medici, D. Molecular and cellular mechanisms of heterotopic ossification. *Histol. Histopathol.* **2014**, *29*, 1281–1285. [PubMed]
185. Aykul, S.; Corpina, R.A.; Goebel, E.J.; Cunanan, C.J.; Dimitriou, A.; Kim, H.J.; Zhang, Q.; Rafique, A.; Leidich, R.; Wang, X.; et al. Activin A forms a non-signaling complex with ACVR1 and type II Activin/BMP receptors via its finger 2 tip loop. *eLife* **2020**, *9*, e54582. [CrossRef]
186. Latres, E.; Mastaitis, J.; Fury, W.; Miloscio, L.; Trejos, J.; Pangilinan, J.; Okamoto, H.; Cavino, K.; Na, E.; Papatheodorou, A.; et al. Activin A more prominently regulates muscle mass in primates than does GDF8. *Nat. Commun.* **2017**, *8*, 15153. [CrossRef]
187. Vanhoutte, F.; Liang, S.; Ruddy, M.; Zhao, A.; Drewery, T.; Wang, Y.; DelGizzi, R.; Forleo-Neto, E.; Rajadhyaksha, M.; Herman, G.; et al. Pharmacokinetics and Pharmacodynamics of Garetosmab (Anti-Activin A): Results from a First-in-Human Phase 1 Study. *J. Clin. Pharm.* **2020**, *60*, 1424–1431. [CrossRef]
188. Yamamoto, R.; Matsushita, M.; Kitoh, H.; Masuda, A.; Ito, M.; Katagiri, T.; Kawai, T.; Ishiguro, N.; Ohno, K. Clinically applicable antianginal agents suppress osteoblastic transformation of myogenic cells and heterotopic ossifications in mice. *J. Bone Miner. Metab.* **2013**, *31*, 26–33. [CrossRef]

189. Kitoh, H.; Achiwa, M.; Kaneko, H.; Mishima, K.; Matsushita, M.; Kadono, I.; Horowitz, J.D.; Sallustio, B.C.; Ohno, K.; Ishiguro, N. Perhexiline maleate in the treatment of fibrodysplasia ossificans progressiva: An open-labeled clinical trial. *Orphanet J. Rare Dis.* **2013**, *8*, 163. [CrossRef]
190. Molinuevo, M.S.; Schurman, L.; McCarthy, A.D.; Cortizo, A.M.; Tolosa, M.J.; Gangoiti, M.V.; Arnol, V.; Sedlinsky, C. Effect of metformin on bone marrow progenitor cell differentiation: In vivo and in vitro studies. *J. Bone Miner. Res.* **2010**, *25*, 211–221. [CrossRef]
191. Jang, W.G.; Kim, E.J.; Bae, I.H.; Lee, K.N.; Kim, Y.D.; Kim, D.K.; Kim, S.H.; Lee, C.H.; Franceschi, R.T.; Choi, H.S.; et al. Metformin induces osteoblast differentiation via orphan nuclear receptor SHP-mediated transactivation of Runx2. *Bone* **2011**, *48*, 885–893. [CrossRef] [PubMed]
192. Lin, H.; Shi, F.; Jiang, S.; Wang, Y.; Zou, J.; Ying, Y.; Huang, D.; Luo, L.; Yan, X.; Luo, Z. Metformin attenuates trauma-induced heterotopic ossification via inhibition of Bone Morphogenetic Protein signalling. *J. Cell. Mol. Med.* **2020**, *24*, 14491–14501. [CrossRef] [PubMed]
193. Yu, P.B.; Hong, C.C.; Sachidanandan, C.; Babitt, J.L.; Deng, D.Y.; Hoyng, S.A.; Lin, H.Y.; Bloch, K.D.; Peterson, R.T. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat. Chem. Biol.* **2008**, *4*, 33–41. [CrossRef] [PubMed]
194. Williams, E.; Bullock, A.N. Structural basis for the potent and selective binding of LDN-212854 to the BMP receptor kinase ALK2. *Bone* **2018**, *109*, 251–258. [CrossRef] [PubMed]
195. Strong, A.L.; Spreadborough, P.J.; Dey, D.; Yang, P.; Li, S.; Lee, A.; Haskins, R.M.; Grimm, P.D.; Kumar, R.; Bradley, M.J.; et al. BMP Ligand Trap ALK3-Fc Attenuates Osteogenesis and Heterotopic Ossification in Blast-Related Lower Extremity Trauma. *Stem Cells Dev.* **2021**, *30*, 91–105. [CrossRef]
196. Mohedas, A.H.; Wang, Y.; Sanvitale, C.E.; Canning, P.; Choi, S.; Xing, X.; Bullock, A.N.; Cuny, G.D.; Yu, P.B. Structure-activity relationship of 3,5-diaryl-2-aminopyridine ALK2 inhibitors reveals unaltered binding affinity for fibrodysplasia ossificans progressiva causing mutants. *J. Med. Chem.* **2014**, *57*, 7900–7915. [CrossRef]
197. Hao, J.; Ho, J.N.; Lewis, J.A.; Karim, K.A.; Daniels, R.N.; Gentry, P.R.; Hopkins, C.R.; Lindsley, C.W.; Hong, C.C. In vivo structure-activity relationship study of dorsomorphin analogues identifies selective VEGF and BMP inhibitors. *ACS Chem. Biol.* **2010**, *5*, 245–253. [CrossRef]
198. Tsugawa, D.; Oya, Y.; Masuzaki, R.; Ray, K.; Engers, D.W.; Dib, M.; Do, N.; Kuramitsu, K.; Ho, K.; Frist, A.; et al. Specific activin receptor-like kinase 3 inhibitors enhance liver regeneration. *J. Pharm. Exp. Ther.* **2014**, *351*, 549–558. [CrossRef]
199. Hino, K.; Zhao, C.; Horigome, K.; Nishio, M.; Okanishi, Y.; Nagata, S.; Komura, S.; Yamada, Y.; Toguchida, J.; Ohta, A.; et al. An mTOR Signaling Modulator Suppressed Heterotopic Ossification of Fibrodysplasia Ossificans Progressiva. *Stem Cell Rep.* **2018**, *11*, 1106–1119. [CrossRef]
200. Kitoh, H. Clinical Aspects and Current Therapeutic Approaches for FOP. *Biomedicines* **2020**, *8*, 325. [CrossRef]
201. Hildebrandt, S.; Kampfrath, B.; Fischer, K.; Hildebrand, L.; Haupt, J.; Stachelscheid, H.; Knaus, P. ActivinA Induced SMAD1/5 Signaling in an iPSC Derived EC Model of Fibrodysplasia Ossificans Progressiva (FOP) Can Be Rescued by the Drug Candidate Saracatinib. *Stem Cell Rev. Rep.* **2021**, *17*, 1039–1052. [CrossRef] [PubMed]
202. Williams, E.; Bagarova, J.; Kerr, G.; Xia, D.D.; Place, E.S.; Dey, D.; Shen, Y.; Bocobo, G.A.; Mohedas, A.H.; Huang, X.; et al. Saracatinib is an efficacious clinical candidate for fibrodysplasia ossificans progressiva. *JCI Insight* **2021**, *6*, e95042. [CrossRef] [PubMed]
203. Pang, J.; Zuo, Y.; Chen, Y.; Song, L.; Zhu, Q.; Yu, J.; Shan, C.; Cai, Z.; Hao, J.; Kaplan, F.S.; et al. ACVR1-Fc suppresses BMP signaling and chondro-osseous differentiation in an in vitro model of Fibrodysplasia ossificans progressiva. *Bone* **2016**, *92*, 29–36. [CrossRef]
204. Kaplan, F.S.; Pignolo, R.J.; Al Mukaddam, M.M.; Shore, E.M. Hard targets for a second skeleton: Therapeutic horizons for fibrodysplasia ossificans progressiva (FOP). *Expert Opin. Orphan Drugs* **2017**, *5*, 291–294. [CrossRef] [PubMed]
205. Kaplan, F.S.; Pignolo, R.J.; Shore, E.M. From mysteries to medicines: Drug development for fibrodysplasia ossificans progressive. *Expert Opin. Orphan Drugs* **2013**, *1*, 637–649. [CrossRef]
206. Cappato, S.; Tonachini, L.; Giacopelli, F.; Tirone, M.; Galietta, L.J.; Sormani, M.; Giovenzana, A.; Spinelli, A.E.; Canciani, B.; Brunelli, S.; et al. High-throughput screening for modulators of ACVR1 transcription: Discovery of potential therapeutics for fibrodysplasia ossificans progressiva. *Dis. Model. Mech.* **2016**, *9*, 685–696. [CrossRef]
207. Tirone, M.; Giovenzana, A.; Vallone, A.; Zordan, P.; Sormani, M.; Nicolosi, P.A.; Meneveri, R.; Gigliotti, C.R.; Spinelli, A.E.; Bocciardi, R.; et al. Severe Heterotopic Ossification in the Skeletal Muscle and Endothelial Cells Recruitment to Chondrogenesis Are Enhanced by Monocyte/Macrophage Depletion. *Front. Immunol.* **2019**, *10*, 1640. [CrossRef]
208. Wang, Z.Q.; Lu, Y.Q.; Han, J.X. MicroRNAs: Important mediators of ossification. *Chin. Med. J.* **2012**, *125*, 4111–4116.
209. Mura, M.; Cappato, S.; Giacopelli, F.; Ravazzolo, R.; Bocciardi, R. The role of the 3′UTR region in the regulation of the ACVR1/Alk-2 gene expression. *PLoS ONE* **2012**, *7*, e50958. [CrossRef]
210. Oishi, T.; Uezumi, A.; Kanaji, A.; Yamamoto, N.; Yamaguchi, A.; Yamada, H.; Tsuchida, K. Osteogenic differentiation capacity of human skeletal muscle-derived progenitor cells. *PLoS ONE* **2013**, *8*, e56641. [CrossRef]
211. Guerit, D.; Philipot, D.; Chuchana, P.; Toupet, K.; Brondello, J.M.; Mathieu, M.; Jorgensen, C.; Noel, D. Sox9-regulated miRNA-574-3p inhibits chondrogenic differentiation of mesenchymal stem cells. *PLoS ONE* **2013**, *8*, e62582. [CrossRef] [PubMed]
212. Sun, Y.; Cai, J.; Yu, S.; Chen, S.; Li, F.; Fan, C. MiR-630 Inhibits Endothelial-Mesenchymal Transition by Targeting Slug in Traumatic Heterotopic Ossification. *Sci. Rep.* **2016**, *6*, 22729. [CrossRef] [PubMed]

213. Qin, X.; Zhu, B.; Jiang, T.; Tan, J.; Wu, Z.; Yuan, Z.; Zheng, L.; Zhao, J. miR-17-5p Regulates Heterotopic Ossification by Targeting ANKH in Ankylosing Spondylitis. *Mol. Nucleic Acids* **2019**, *18*, 696–707. [CrossRef] [PubMed]
214. Liu, N.; Zhang, Z.; Li, L.; Shen, X.; Sun, B.; Wang, R.; Zhong, H.; Shi, Q.; Wei, L.; Zhang, Y.; et al. MicroRNA-181 regulates the development of Ossification of Posterior longitudinal ligament via Epigenetic Modulation by targeting PBX1. *Theranostics* **2020**, *10*, 7492–7509. [CrossRef] [PubMed]
215. Aartsma-Rus, A.; Fokkema, I.; Verschuuren, J.; Ginjaar, I.; van Deutekom, J.; van Ommen, G.J.; den Dunnen, J.T. Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. *Hum. Mutat.* **2009**, *30*, 293–299. [CrossRef]
216. Shi, S.; Cai, J.; de Gorter, D.J.; Sanchez-Duffhues, G.; Kemaladewi, D.U.; Hoogaars, W.M.; Aartsma-Rus, A.; 't Hoen, P.A.; ten Dijke, P. Antisense-oligonucleotide mediated exon skipping in activin-receptor-like kinase 2: Inhibiting the receptor that is overactive in fibrodysplasia ossificans progressiva. *PLoS ONE* **2013**, *8*, e69096. [CrossRef]
217. Shi, F.; Gao, J.; Zou, J.; Ying, Y.; Lin, H. Targeting heterotopic ossification by inhibiting activin receptorlike kinase 2 function (Review). *Mol. Med. Rep.* **2019**, *20*, 2979–2989.
218. Kaplan, J.; Kaplan, F.S.; Shore, E.M. Restoration of normal BMP signaling levels and osteogenic differentiation in FOP mesenchymal progenitor cells by mutant allele-specific targeting. *Gene Ther.* **2012**, *19*, 786–790. [CrossRef]
219. Shrivats, A.R.; Hsu, E.; Averick, S.; Klimak, M.; Watt, A.C.; DeMaio, M.; Matyjaszewski, K.; Hollinger, J.O. Cationic Nanogel-mediated Runx2 and Osterix siRNA Delivery Decreases Mineralization in MC3T3 Cells. *Clin. Orthop. Relat. Res.* **2015**, *473*, 2139–2149. [CrossRef]
220. Patil, S.; Dang, K.; Zhao, X.; Gao, Y.; Qian, A. Role of LncRNAs and CircRNAs in Bone Metabolism and Osteoporosis. *Front. Genet.* **2020**, *11*, 584118. [CrossRef]
221. Ghafouri-Fard, S.; Abak, A.; Avval, S.T.; Rahmani, S.; Shoorei, H.; Taheri, M.; Samadian, M. Contribution of miRNAs and lncRNAs in osteogenesis and related disorders. *Biomed. Pharmacother.* **2021**, *142*, 111942. [CrossRef] [PubMed]
222. Liu, L.; Li, Z.; Chen, S.; Cui, H.; Li, X.; Dai, G.; Zhong, F.; Hao, W.; Zhang, K.; Liu, H. BRD4 promotes heterotopic ossification through upregulation of LncRNA MANCR. *Bone Jt. Res.* **2021**, *10*, 668–676. [CrossRef] [PubMed]