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Review

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Advancements in mechanisms and drug treatments for fibrodysplasia ossificans progressiva

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Abstract: Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder characterized by congenital bilateral malformation of the large toe and progressive, extensive, and irreversible heterotopic ossification (HO) of soft tissues throughout the body, leading to severe disabilities. FOP is caused primarily by mutations in activin A receptor type 1 (*ACVR1*), also known as activin-like kinase 2 (*ALK2*), which encodes a receptor belonging to the bone morphogenetic protein (BMP) type I family. However, the continuous and complex process of HO in FOP is not yet fully understood, which has impeded the development of therapeutic drugs. Despite surgical removal of HO, which often results in recurrence and expansion of ossification, there is currently no definitive drug treatment available to completely prevent, halt, or reverse the progression of HO in FOP. Currently, researchers are intensively studying the pathogenesis of FOP at various stages and developing promising drug candidates, including saracatinib, palovarotene, and rapamycin. This review provides an overview of progress in understanding the mechanism of FOP and the development of therapeutic drugs, with the goal of providing insights for further research and the development of new treatment methods.

Key words: Fibrodysplasia ossificans progressiva (FOP); Heterotopic ossification (HO); Mechanism; Drug treatment; Activin A receptor type 1 (ACVR1)

1 Introduction

Fibrodysplasia ossificans progressiva (FOP, OMIM135100) is a rare genetic heterotopic ossification (HO) disease (Towler and Shore, 2022) with a prevalence of about 1 in 2 million individuals in the Western world (She and Zhang, 2018). It is characterized by congenital bilateral malformation of the large toe presenting as short, thick, and valgus, and by extensive, progressive, and irreversible HO of the soft tissues throughout the body. Even minor local inflammation, such as vaccination, can cause HO. Progressive HO leads to trunk deformities and joint contractures in the extremities, and systemic ankyloses can result in walking difficulties and respiratory dysfunction in some patients (Kitoh, 2020), significantly impairing both their quality of life and life expectancy. The life expectancy of patients is generally only about 40 years (Pignolo et al., 2022a).

Currently, there is no definitive drug treatment available to completely prevent, halt, or reverse HO in FOP, and trauma prevention remains a primary therapeutic focus (Kitoh, 2020). Glucocorticoids, nonsteroidal anti-inflammatory drugs (NSAIDs), leukotriene inhibitors, and mast cell stabilizers are used to control inflammation (Brennan et al., 2018; Wentworth et al., 2019). However, the effect of current drug therapy is limited, leading to recurrence and expansion of ossification after surgical removal of HO. Moreover, the three main current requirements for clinical medicine, namely (1) drugs that can be safely used to prevent HO on a daily basis, (2) managing pain, and (3) removing/reducing the HO that has already formed to relieve fused joints, are not being adequately met.

Research has shown that all reported FOP cases show mutations in the activin A receptor type 1 (*ACVR1*)

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gene and 95% show the *ACVR1*^{R206H} (c.617G>A;p.R206H) mutation (Shore et al., 2006), resulting in ACVR1 becoming an activin receptor and activating the downstream SMAD family member 1/5/9 (SMAD1/5/9) signaling pathway (Hatsell et al., 2015). However, HO in FOP is a complex process. This review provides an overview of the research focused on the pathogenesis of FOP and the development of promising drug candidates for further research.

2 Mutation of the *ACVR1* gene and changes in TGF-β/BMP signaling pathways

2.1 Mechanisms

FOP is caused by mutations in the *ACVR1* gene at 2q23–24 (Shore et al., 2006). ACVR1 is a bone morphogenetic protein (BMP) type I transmembrane serine/threonine kinase receptor. BMP type I receptors, which include ACVR1 (activin-like kinase 2 (ALK2)), BMPR1A (ALK3), and BMPR1B (ALK6), can form heterotetramers with BMP type II receptors such as BMPR2, ACVR2A, and ACVR2B upon BMP binding (Hu et al., 2021). BMP type II receptors subsequently phosphorylate the glycine/serine region of BMP type I receptors, triggering downstream signaling pathways (Jia and Meng, 2021). The *ACVR1*^{R206H} mutation results in aberrant function of its protein as an activin receptor, leading to perpetual activation of the downstream SMAD1/5/9 signaling pathway (Hatsell et al., 2015). This mutation also reduces the binding affinity of ACVR1 to its negative regulator the 12-kDa FK506-binding protein (FKBP12), thereby promoting the onset and progression of HO in FOP (Song et al., 2010; Hatsell et al., 2015) (Fig. 1).

Activin A (ACTA) is a secreted factor implicated in the development of HO in FOP (Fig. 1). Experiments involving *Acvr1*$^{[R206H]FlEx/+}$ transgenic mice as an FOP model have shown that these mice do not develop HO either spontaneously or in response to local injury once the activation of ACVR1 by ACTA is blocked (Hatsell et al., 2015). While some researchers have reported increased ACTA production in fibroblasts from FOP patients (de Ruiter et al., 2022), others have found no significant differences in serum ACTA levels between FOP patients and healthy controls (Ye et al., 2023). This discrepancy suggests a potential tissue/cell-specific effect of ACTA that warrants further investigation.

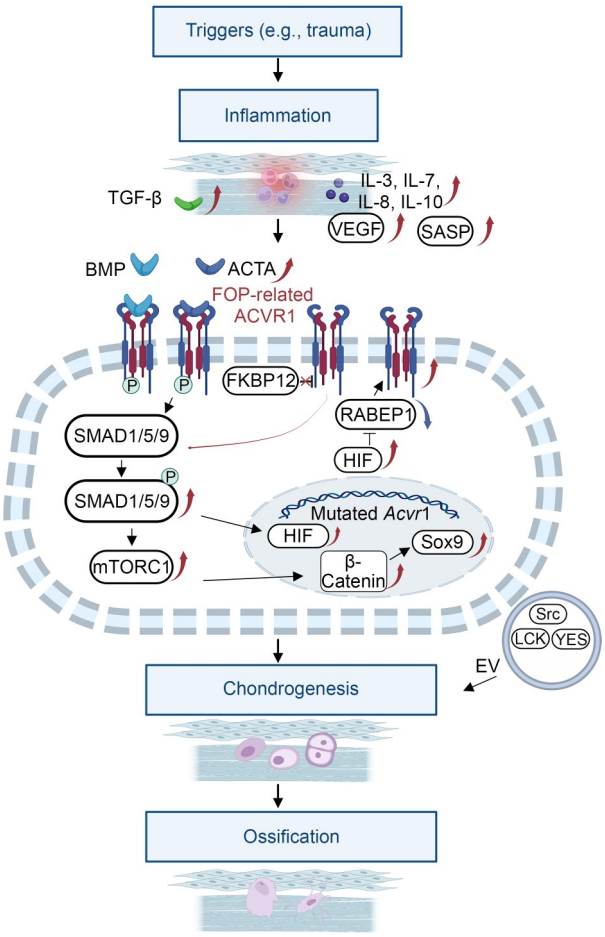

Fig. 1 Schematic overview of currently known fibrodysplasia ossificans progressiva (FOP) mechanisms (created with BioRender.com). ACTA: activin A; ACVR1: activin A receptor type 1; BMP: bone morphogenetic protein; EV: extracellular vesicle; FKBP12: 12-kDa FK506-binding protein; HIF: hypoxia-inducible factor; IL: interleukin; LCK: lymphocyte-specific protein-tyrosine kinase; mTORC1: mechanistic target of rapamycin complex 1; P: phosphorylation; RABEP1: Rab GTPase-binding effector protein 1, Rabaptin 5; SASP: senescence-associated secretory phenotype; SMAD: mothers against decapentaplegic homolog; Sox9: sex-determining region Y (SRY)-box 9; Src: steroid receptor coactivator; TGF-β: transforming growth factor-β; VEGF: vascular endothelial growth factor; YES: YES proto-oncogene.

ACTA has been shown to either engage ACVR1B to activate SMAD2/3 or bind ACVR1 and activin/BMP type II receptors via its finger 2 tip loop to form a non-signaling complex (NSC) in the BMP signaling pathway. FOP-associated *ACVR1* missense mutations can convert this NSC into a signaling complex. The NSC containing wild-type (WT) ACVR1 exerts a negative regulatory effect on the signaling that drives HO in FOP (Aykul et al., 2020), and overexpression of WT

Acvr1 in FOP mice inhibits HO (Yamamoto et al., 2022). Overexpression of *ACVR1* in fibro-adipogenic progenitors (FAPs) has a cell-autonomous effect, reducing ACTA-induced osteogenic signaling (Yamamoto et al., 2022). These findings underscore a regulatory mechanism of the levels of BMP signaling abnormality induced by ACTA with the $ACVR1^{R206H}$ mutation.

In addition to ACVR1 and ACTA, other molecules in the BMP pathway have attracted research interest. For instance, in zebrafish, Wentworth et al. (2022) found that the knockdown of WT *BMPR1A* and *ACVR2A* could weaken $ACVR1^{R206H}$ signaling, particularly in response to ACTA. FOP also implicates signaling cascades within the transforming growth factor-β (TGF-β) superfamily. Notably, TGF-β/BMP family ligands can also transmit signals via non-SMAD cascades, such as TGF-β-activated kinase 1 (TAK1) (Zhang, 2017; Hwang et al., 2022). In FOP patients, macrophages have been observed to secrete increased amounts of TGF-β and exhibit prolonged nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK) activation without phosphorylated SMAD1/5 (p-SMAD1/5) changes, indicating dysregulated TAK1 activation (Barruet et al., 2018). Recent research has shown that in primary dermal fibroblasts from FOP patients, TGF-β1 acted as an FOP-specific stimulant of ACTA, as shown by the upregulation of inhibin subunit βA (INHBA) expression (de Ruiter et al., 2023). Therefore, TGF-β signaling pathways may play a previously unexpected role in FOP, and we expect further insights from ongoing research.

2.2 Drug treatments

2.2.1 Gene therapy

Gene therapy, a promising avenue for diseases with genetic origins such as inherited retinal dystrophy (Chiu et al., 2021), is similarly being explored for FOP. Several gene therapy approaches are under investigation for the treatment of FOP due to the highly consistent genetic etiology, the severe lifetime progression of ectopic ossification, and the heavy burden of lifetime medication.

2.2.1.1 Small interfering RNA (siRNA)

Kaplan et al. (2012) used allele-specific RNA interference (ASP-RNAi) to suppress disease-related alleles without inhibiting the normal alleles and reported that ASP-RNAi could mediate selective suppression of the mutant c.617A*ACVR1*, decrease the increased BMP pathway signaling, and restore enhanced osteogenic differentiation of FOP SHED cells. Yang et al. (2022) used a combined approach of gene replacement and silencing, by an adeno-associated virus (AAV)-compatible artificial microRNA, to suppress ACTA-induced aberrant BMP signaling of human FOP-induced pluripotent stem cells (iPSCs) and mouse $Acvr1^{(R206H)KI}$ skeletal cells. This approach prevented both traumatic and spontaneous HO in $Acvr1^{(R206H)KI}$ FOP mice without causing detrimental effects on cartilage development, bone growth, or bone remodeling. They also tried AAV combinations targeting ACVR1 and ACTA, and the local delivery of this AAV gene therapy resulted in a significant decrease in endochondral bone formation in $Acvr1^{R206H/+}$ mice (Yang et al., 2023). We expect further insights concerning optimization of the combination, vector biodistribution, toxicity, dose-ranging, and especially side effects.

2.2.1.2 Antisense oligonucleotides (ASOs)

ASOs are synthetic single-stranded DNA or RNA sequences, typically 15–25 nucleotides in length, that downregulate gene expression by inducing RNaseH-mediated degradation of the target transcript (gapmers) or preventing the binding of proteins to RNA (steric-blocking) (Lim et al., 2020). Recently, the US Food and Drug Administration (FDA) approved nusinersen, an ASO, for the treatment of spinal muscular atrophy (Nagar et al., 2023).

Shi et al. (2013) designed ASOs targeting exon 8 of the WT mouse *Acvr1* gene. These ASOs knocked down mouse *Acvr1* expression through exon skipping, promoting myogenic differentiation of C2C12 myoblasts and inhibiting BMP6-induced osteoblast differentiation in mouse endothelial cells (ECs).

Maruyama and Yokota (2018) created a phosphorodiamidate morpholino oligomer (PMO), a morpholine moiety connected through methylene phosphorodiamidate that replaces the ribose, and successfully skipped the FOP mutation (exon 6) of *ACVR1* in FOP patient fibroblasts.

Building on ASO technology, locked nucleic acids (LNAs) offer increased stability by connecting the 2'-oxygen with the 4'-carbon on the same ribose through a methylene bridge (Xiong et al., 2021). Maruyama et al. (2022) developed LNA gapmers, short DNA oligonucleotides with LNA modifications at both ends, which efficiently knocked down $ACVR1^{R206H}$ expression at the RNA level, while $ACVR1^{WT}$ was mainly unaffected in human FOP fibroblasts. Furthermore, some LNA gapmers suppressed ACTA-induced osteogenic differentiation in C2C12 ($Acvr1^{R206H}$) cells.

While this strategy shows promise for modulating the activity of the *ACVR1* gene, its efficacy in FOP mouse models remains to be studied.

2.2.2 Inhibitors of BMP type I receptor/ACVR1

A common strategy in developing FOP drugs is to inhibit the function of ACVR1 or the BMP type I receptor. The first inhibitor discovered was dorsomorphin, identified during in vivo screening of BMP inhibitors (Yu et al., 2008b). Dorsomorphin exerts its inhibitory effect on ACVR1 by directly binding to the adenosine triphosphate (ATP)-binding pocket of ACVR1's intracellular kinase domain through its pyrazolo[1,5-*a*] pyrimidine core (Chaikuad et al., 2012). However, concerns regarding its clinical safety arose due to the poor specificity of dorsomorphin (Luo et al., 2016). Subsequently, more selective inhibitors, namely DMH1 (Hao et al., 2010) and LDN-193189 (Cuny et al., 2008; Yu et al., 2008a), were developed and shown to be effective in FOP animal models. To better distinguish ACVR1 (ALK2) from other BMP type I receptors (ALK1/ACVRL1, ALK3/BMPR1A, and ALK6/BMPR1B) and inhibit ACVR1 more specifically while reducing side effects, a series of inhibitors were developed, including LDN-212854 (Mohedas et al., 2013), ML347 (Engers et al., 2013), OD36 (Sánchez-Duffhues et al., 2019), and "Compound 23" (Ullrich et al., 2022).

Among these modified compounds, LDN-212854 exhibits enhanced specificity in targeting ACVR1 (Mohedas et al., 2013) and has undergone extensive research. LDN-212854 was developed from LDN-193189, and the modification increases the specificity for ACVR1 (ALK2) over BMPR1A (ALK3) by 66 times and the specificity for ACVR1 over ALK5 by over 9000 times (Mohedas et al., 2013). LDN-212854 was shown to inhibit the formation of HO in FOP mouse models which are transgenic mice with inducible constitutively active ACVR1 (caACVR1) (*Acvr1*^{Q207D}) (Mohedas et al., 2013; Dey et al., 2016). In vitro studies revealed that LDN-212854 can partly inhibit the mineralization of pericytes from human iPSCs (hiPSCs) of FOP patients with the *ACVR1*^{R206H} mutation (Cai et al., 2015). However, under the same drug concentration (6 mg/kg, intraperitoneal injection (IP), twice daily), there was no significant difference in the range of motion score between LDN-212854 and LDN-193189 in caACVR1 mice (Mohedas et al., 2013). These results indicate that the difference in inhibition specificity between the two compounds is not significant in inhibiting HO formation in FOP, but may have some significance in reducing side effects.

Sánchez-Duffhues et al. (2019) used a chemistry-based platform of macrocyclic kinase inhibitors and FOP-derived ECs to develop drugs targeting ACVR1. They found that OD36 and OD52 displayed improved specificity compared with LDN-193189, efficiently inhibited BMP-6 induced phosphorylation of SMAD1/5/9 in KS483 cells, exclusively inhibited caACVR1 in C2C12 cells, and inhibited ACTA-induced osteogenic and chondrogenic differentiation of blood-derived endothelial colony-forming cells (ECFCs) from *ACVR1*^{R206H} FOP donors.

Jiang et al. (2018) identified a new ACVR1 inhibitor "Compound 23" with 4(sulfamoyl)naphthyl as the pendant substituent at the 3-position of the pyrazolo[1,5-*a*]pyrimidine core, which exhibited better specificity for ACVR1 compared with other BMP type I receptor kinases and showed acceptable in vivo pharmacokinetics.

In addition to the pyrazolo[1,5-*a*]pyrimidine scaffold, some researchers identified the imidazo[1,2-*a*] pyridine core as a suitable replacement system for potent ALK inhibitors and explored ways for improving its metabolic stability. As a result, "Compound 2" was developed, which has a specificity profile similar to that of LDN-193189, good oral bioavailability in rats, and a favorable brain plasma ratio of 1.6 (Engers et al., 2020).

On the other hand, through screening 250 recombinant human kinases, Sanvitale et al. (2013) identified K02288 with a pyridine core, showing in vitro activity against ACVR1 at low nanomolar concentrations similar to LDN-193189. K02288 specifically inhibited the BMP-induced SMAD pathway without affecting TGF-β signaling (Sanvitale et al., 2013). However, similar to LDN-193189, K02288 shows low specificity for ACVR1 versus BMPR1A and ALK5 (Mohedas et al., 2013).

Ullrich et al. (2022) discovered several compounds with strikingly similar structural characteristics to K02288 analogs and detailed the optimization of a novel series of ACVR1 inhibitors based on a 2-aminopyrazine-3-carboxamide scaffold. Among these compounds, "Compound 23" demonstrated high aqueous solubility, membrane permeability, and microsomal stability, along with biochemical and cellular potency. It also showed good selectivity over other BMP and TGF-β receptor kinases.

Sato et al. (2020) reported a series of *bis*-heteroaryl pyrazoles as ACVR1 inhibitors, including RK-59638. Based on RK-59638, "Compound 8" was reported to bind to the ATP-binding pocket of ACVR1^{R206H}, showing good membrane permeability and a strong ability to inhibit alkaline phosphatase (ALP) activity in C2C12 (*Acvr1*^{R206H}) cells (Yamamoto et al., 2021).

Witten et al. (2022) designed a novel series of potent and selective ACVR1 inhibitors based on a bicyclic lactam scaffold. Their specific roles in cells remain to be studied.

Through in-house high-throughput screening and structure-guided drug design, Nguyen et al. (2022) recently discovered a series of pyrazolopyrimidines that act as selective ALK2 inhibitors. Among these, "Compound 16" showed high selectivity against other ALK subtypes, a favorable pharmacokinetic profile, and significant in vivo suppression of hepcidin which is regulated by ALK2 in hepatocytes.

Saracatinib, also known as AZD0530, is an oral steroid receotor coactivator (Src) inhibitor developed by AstraZeneca for the treatment of ovarian adenocarcinoma (Hennequin et al., 2006), with favorable pharmacokinetic parameters and a suitable safety profile at a daily dosage of up to 250 mg (Hannon et al., 2010). Williams et al. (2021) screened a library of clinically tested small-molecule inhibitors and found that saracatinib showed a good specific inhibitory effect on ACVR1. Saracatinib has been shown to inhibit the formation of HO in the *Acvr1*^{Q207D} and *Acvr1*^{R206H} transgenic mouse models of FOP (Williams et al., 2021). Following treatment with saracatinib, the transcriptome induced by ACTA in an FOP-induced EC (iEC) model generated from iPSCs was rescued to WT levels. However, the transcription of *SMAD2/3* target genes induced by ACTA was preserved in both WT iECs and FOP-iECs (Hildebrandt et al., 2021). Saracatinib is currently undergoing a Phase II clinical trial for FOP (registered under NCT04307953) with a daily dose of 100 mg administered over a treatment period of 6 to 18 months.

To specifically target FOP-mutated *ACVR1* without affecting WT *ACVR1*, Blueprint Medicines Inc. developed IPN60130 (also known as BLU-782) (Meng et al., 2022). In a Phase I clinical trial of IPN60130 in healthy volunteers (NCT03858075), the drug demonstrated good tolerability and favorable pharmacokinetic and pharmacodynamic properties (Blueprint Medicines Corporation, 2019). IPN60130 is currently in Phase II clinical trials (NCT05039515).

INCB000928 is a potent and orally bioavailable small molecule inhibitor, showing high specificity for ACVR1 over ACVRL1 and BMPR1A in biochemical enzyme assays. INCB00928 also showed favorable pharmacokinetic properties in in vivo rodent studies. Serine/threonine kinase and ACVR1 are known to regulate serum ferrimodulin levels. Knockdown or loss of *Acvr1* in preclinical animal studies has been shown to result in decreased ferrimodulin production and elevated serum iron levels. INCB00928 inhibits BMP-induced ferrimodulin expression in both immortalized human liver cell lines and primary human liver cells. Furthermore, it reduces the levels of p-SMAD1/5 in the liver tissues of drug-treated mice (Chen et al., 2020). These findings suggest that INCB000928 holds promise as a potential treatment for FOP, and Phase II clinical trials for FOP (NCT05090891) are currently underway (Incyte Corporation, 2024).

Several research groups are working to identify natural inhibitors of ACVR1, often derived from marine organisms. For instance, Ohte et al. (2021) isolated a new diketopiperazine-like compound, named protuboxepin K, from the culture broth of the marine-derived fungal strain *Aspergillus* sp. BFM-0085. They also identified a unique sesquiterpene lactone, bicyclolamellolactone A, from the Indonesian marine sponge *Lamellodysidea* sp. (cf. *L. herbacea*). Similarly, Yamazaki et al. (2020) isolated dysidenin, herbasterol, and stellettasterol from ethanol extracts of the marine sponge *Dysidea* sp. (No. 256), following the screening of extracts from 188 Indonesian marine invertebrates. All these compounds demonstrated potent ALP inhibitory activity in C2C12 (*Acvr1*^{R206H}) cells. However, the specificity and effectiveness of these natural inhibitors in FOP mouse models are unknown. Nonetheless, the discovery of new natural inhibitors may provide new insights into the mechanism of action of ACVR1.

Several related inhibitors have shown their effectiveness in preclinical studies, while the results from other inhibitors developed or identified more recently are yet to be disclosed.

2.2.3 Anti-ACVR1 antibodies

Building on the concept of blocking ACVR1, Daiichi Sankyo is developing the anti-ACVR1 monoclonal antibody DS-6016a in collaboration with Saitama Medical University in Japan. A Phase I clinical trial of DS-6016a has recently been completed

(NCT04818398), confirming the safety, tolerability, and pharmacokinetics of DS-6016a in healthy Japanese participants (IFOPA, 2022). However, specific experimental results for DS-6016a are not yet available.

Another anti-ACVR1 monoclonal antibody, Rm0443, was isolated from a rat hybridoma cell line. This antibody binds to mouse and human ACVR1 through the residue H64, selectively inhabits ACVR1 signaling, and prevents HO in BMP7-induced HO mouse models and in one FOP mouse model (*hALK2*$^{R206H\ FlEx\ KI}$ CAG-cre/Esr1). However, it increased HO in another FOP mouse model (*mAlk2*$^{R206H\ FlEx\ KI}$). The contrasting effects of Rm0443 on human and mouse ACVR1 are attributed to a single residue difference at position 330. As Rm0443 is a rat antibody, researchers have developed humanized antibodies, including the aforementioned DS-6016a (Katagiri et al., 2023).

Conversely, some studies have reported that certain anti-ACVR1 antibodies exacerbated HO in FOP (*Acvr1*^{R206H}) mouse models, even though the Fab fragments of these antibodies still inhibited ACVR1 signaling in vitro (Aykul et al., 2022; Lees-Shepard et al., 2022). In view of these contradictory in vivo results, it is speculated that the divalent antibodies for type I receptors prompt dimer formation to activate BMP signaling, which explains why the monovalent Fab fragment inhibits ACVR1 signaling in vitro as expected. The single residue difference at position 330 between human and mouse ACVR1 may provide clues to support this. Therefore, further studies are needed to elucidate the blocking mechanisms of anti-ACVR1 antibodies with ACVR1 from different species.

2.2.4 Anti-ACTA antibodies

As previously mentioned, ACTA, a secreted factor, is essential for the development of HO in FOP. Studies conducted on *Acvr1*$^{[R206H]FlEx/+}$ transgenic mice used as an FOP model demonstrated that these mice did not develop HO either spontaneously or in response to local injury when treated with antibodies against ACTA. These antibodies block the activation of ACVR1 by ACTA (Hatsell et al., 2015). Consequently, ongoing research is exploring the potential of anti-ACTA antibodies for the treatment of FOP (Fig. 2).

Research has shown that treatment with Regeneron anti-ACTA antibody, garetosmab (also known as REGN247711), significantly inhibits HO in the FOP mouse model. Moreover, it not only inhibits the development of HO after the formation of bone tissue but also promotes the resorption of pre-existing heterotopic bone tissue to some extent (Upadhyay et al., 2017). According to reports, this antibody has recently completed a Phase II clinical trial (NCT04307953). FOP patients enrolled in the 28-week garetosmab treatment trial exhibited a 25% reduction in overall heterotopic bone tissue and nearly 90% reduction in newly developed heterotopic bone tissue compared to patients treated with placebo. However, further research and analysis are needed to address the occurrence of serious adverse events, such as pneumonia and abscess, during the trial period (Ventura et al., 2021).

2.2.5 TGF-β-related inhibitors

Given the established role of the TGF-β signaling pathway in FOP, several inhibitors of the pathway have been explored. NG-25, a small molecule inhibitor of TAK1, was evaluated and proven to limit heterotopic bone formation in a rat blast-associated trauma HO model and a murine burn tenotomy injury model (Strong et al., 2020). Similarly, galunisertib (also known as LY2157299), a TGF-β receptor I kinase inhibitor that has shown good tolerability in patients with malignant tumors, has been demonstrated to prevent HO development in an acquired HO model (Mao et al., 2022). However, their efficacy in FOP remains to be studied and the results of relevant clinical studies are still to be disclosed.

3 Inflammation in FOP

3.1 Mechanisms

FOP lesions can form spontaneously or be triggered by inflammation or trauma. In the early stages of FOP, macrophages and mast cells infiltrate the flare-up sites. Depletion of these cells has been shown to impair HO in an FOP (*Acvr1*^{R206H}) mouse model (Convente et al., 2018). However, other studies have shown that monocyte depletion, using liposome-encapsulated clodronate in injured skeletal muscle, can actually enhance the contribution of iECs to chondrogenesis and HO formation (Tirone et al., 2019). While the mechanisms of HO formation may vary between different subclasses, the heterogeneity of macrophages in FOP is an issue that requires further investigation.

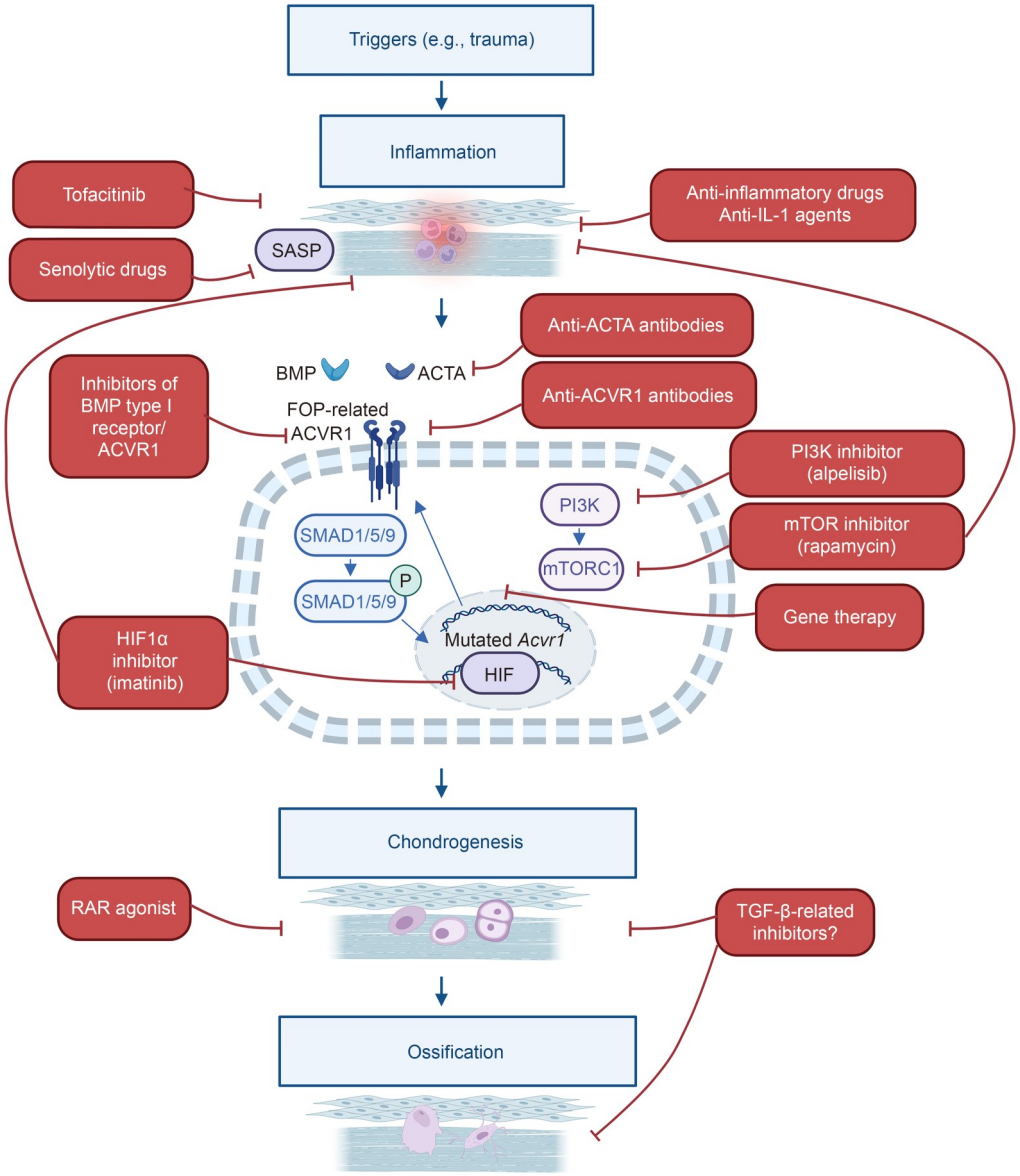

Fig. 2 Schematic overview of the drugs and compounds currently used and/or evaluated in the treatment of fibrodysplasia ossificans progressiva (FOP), and their respective effects (created with BioRender.com). ACTA: activing A; ACVR1: activin A receptor type I; BMP: bone morphogenetic protein; HIF1α: hypoxia-inducible factor 1α; IL: interleukin; mTOR: mammalian target of rapamycin; mTORC1: mTOR complex 1; P: phosphorylation; PI3K: phosphoinositide 3-kinase; RAR: retinoic acid receptor; SASP: senescence-associated secretory phenotype; SMAD: mothers against decapentaplegic homolog; TGF-β: transforming growth factor-β.

As previously mentioned, macrophages in FOP patients exhibit increased secretion of TGF-β and prolonged activation of NF-κB and p38 MAPK, with no significant changes in p-SMAD1/5. Barruet et al. (2018) reported increased levels of interleukin-3 (IL-3), IL-7, IL-8, and IL-10 cytokines in FOP. Hrkac et al. (2022) compared the proteomic profiles of extracellular vehicles (EVs) derived from pooled plasma of FOP patients during flare-up and remission phases. They found that HO occurred via processes of innate immunity and the Ephrin B signaling pathway, which may contribute to HO through macrophage chemotaxis and activation (Fig. 1). Haviv et al. (2019) observed a marked increase in IL-1β plasma levels during "flare-ups" in FOP patients. del Zotto et al. (2018) characterized peripheral blood mononuclear cells (PBMCs) by immunophenotyping in FOP and suggested that the increased expression of DNAX accessory molecule-1 (DNAM1,

also known as CD226) in FOP monocytes may indicate an upregulation of monocyte migration towards inflammation sites during "flare-ups." A case-control study reported correlations between FOP genotype, acute flare-up status, and levels of adiponectin, tenascin-C, and kallikrein-7, suggesting a complex interplay between acute inflammation, chronic inflammation, and genetic background in FOP (Pignolo et al., 2022b).

Using RNA sequencing (RNA-seq), Schoenmaker et al. (2023) described the transcriptomic changes induced by ACTA during osteoclast formation in CD14-positive monocytes from healthy controls and FOP patients. They found that "inflammatory response" (GO: 0006954) was one of the Gene Ontology (GO) terms most significantly associated with upregulated genes, and the class II major histocompatibility complex transactivator (CIITA) was one of the molecules associated with this term. The researchers speculated that ACTA-induced upregulation of CIITA may drive osteoclasts towards a more immunological role under high local concentrations of ACTA, but the specific significance of this finding remains to be explored (Schoenmaker et al., 2023).

In addition, mechanistic target of rapamycin kinase (mTOR) signaling has been shown to play a crucial role in the early inflammatory phase of HO and is required for chondrogenesis and osteogenesis (Hino et al., 2018). Hypoxia, a known promoter of FOP, increases the intensity and duration of canonical BMP signaling through Rabaptin 5 (Rab GTPase-binding effector protein 1 (RABEP1))-mediated retention of ACVR1 in FOP connective tissue progenitor cells (CTPCs) (Wang et al., 2016). These findings suggest potential targets for new treatments.

3.2 Drug treatments

3.2.1 Anti-inflammatory drugs

Given the above-mentioned findings, anti-inflammatory drugs represent the cornerstone of current FOP treatment. High-dose corticosteroids are considered a preventative measure following traumatic injury or prior to unavoidable surgery, and prompt treatment following the first flare-up in a major joint is recommended (Smilde et al., 2022). Nonsteroidal anti-inflammatory drugs (NSAIDs) are also frequently used in FOP, as levels of prostaglandins are elevated in FOP patients experiencing a flare-up (Smilde et al., 2022) (Fig. 2). However, results from clinical trials of these drugs in FOP are still lacking.

FOP also occurs in cats. A recent case report indicated that a regimen of 5 mg/kg twice a day (BID) enrofloxacin and hydrotherapy helped a cat with FOP-like manifestation demonstrate consistent improvement in endurance, quality of life, and range of motion over a period of three years. However, the cat's ACVR1 sequence was not determined, so researchers could not definitively diagnose it with FOP. Regrettably, the cat ultimately succumbed to sudden death (Jacobsen et al., 2023).

3.2.2 Anti-IL-1 agents

In a case report, Haviv et al. (2019) diagnosed a boy with FOP who did not respond to anti-inflammatory therapy. Upon detecting elevated IL-1β plasma levels during a "flare-up," they administered the anti-IL-1 agents anakinra and canakinumab. This treatment reduced the frequency of paroxysmal episodes and shortened their duration (Fig. 2). However, there is still a relative lack of basic research data on this approach.

3.2.3 mTOR inhibitor rapamycin

Rapamycin, a potent and specific inhibitor of mTOR, is commonly used to prevent transplant rejection and lymphangioleiomyomatosis (Ventura et al., 2021). Hino et al. (2017) identified rapamycin as a candidate drug for FOP using an in vitro screening model and induced mesenchymal stromal cells derived from FOP-iPSCs (FOP-iMSCs) to screen for chemical compounds that could suppress the enhanced chondrogenesis of cells. Rapamycin has been reported to suppress HO in several FOP mouse models, including *ACVR1*^{R206H} and *ACVR1*^{Q207D} transgenic mice, as well as in mice with FOP-iPSC transplantation (Hino et al., 2017) and trauma-induced HO mice (Hino et al., 2018) (Fig. 2).

However, recent studies showed that rapamycin did not show clear benefits in reducing HO in two young patients with the *ACVR1*^{R206H} mutation (Kaplan et al., 2018b). It was reported that they continued to develop new flares during rapamycin treatment. However, assessing the response to therapy is challenging since the therapeutic dose may not have been sufficient. Currently, rapamycin is undergoing clinical trials (UMIN000028429) to evaluate its efficacy and safety in patients with FOP, and the outcomes have not been publicly released.

3.2.4 HIF1α inhibitors

Based on studies of the hypoxia-inducible factor (HIF) signaling pathway in FOP, several HIF1α inhibitors, such as apigenin, PX-478, and imatinib, have been reported to prevent HO in FOP mouse models (Wang et al., 2016; Zitvogel et al., 2016) (Fig. 2).

Imatinib is a tyrosine kinase inhibitor that was approved in the 1990s for the treatment of chronic myelogenous leukemia (Capdeville et al., 2002). The drug is well-tolerated with minimal side effects (Hochhaus et al., 2017). Wang et al. (2016) confirmed its effectiveness in inhibiting BMP signaling in SHED cells from FOP patients and in reducing the formation of HO in the caACVR1 FOP mouse model. As a result, imatinib was used off-label in seven children who had severe FOP with continuous flare-ups and were refractory to standard-of-care treatments (Kaplan et al., 2018a). A decrease in the intensity of flare-ups was reported in six of the patients who regularly took imatinib. However, since this was not a randomized controlled trial, clinical trials are needed to provide further evidence regarding the effectiveness of imatinib in preventing HO.

3.2.5 Other related drugs

Some researchers have considered FOP to be an autoinflammatory disease and have explored the potential effectiveness of disease-modifying antirheumatic drugs (DMARDs) for FOP treatment. One such drug is tofacitinib, a Janus kinase (JAK) inhibitor approved by the FDA for the treatment of polyarticular juvenile idiopathic arthritis (JIA). In a retrospective observational study, Nikishina et al. (2023) gathered data from 13 genetically confirmed FOP patients who were treated with tofacitinib at a dosage of 5 mg BID. They found that tofacitinib reduced the number of flares, improved the range of motion in large joints, and improved imaging characteristics.

4 Other mechanisms and drug treatments in FOP

4.1 Mechanisms

4.1.1 Vascularization in FOP

The roles of blood vessels and associated signaling pathways in promoting trauma-induced HO are well-established (Hwang et al., 2019), and histologic images of hematoxylin and eosin (HE)-stained sections from FOP patients demonstrate a richer vascular environment than that seen in nonhereditary HO (Ware et al., 2019). Monocytes from FOP patients have been shown to secrete increased levels of vascular endothelial growth factor (VEGF) in response to inflammation, compared with controls (Tirone et al., 2019) (Fig. 1). Using the previously mentioned FOP-iECs, RNA-seq analysis revealed that ACTA-induced p-SMAD1/5 was upregulated exclusively in the FOP-iECs. This resulted in an FOP-specific transcriptome characterized by the involvement of genes in blood vessel formation and the activation of BMP and NOTCH pathways (Hildebrandt et al., 2021). As a result, vascularization has become a new area of focus in FOP research.

4.1.2 Chondrogenesis and endochondral ossification in FOP

Chondrogenesis and endochondral ossification represent one of the primary mechanisms of HO in FOP. Following muscle injury in FOP mice, a subset of FAPs organize and undergo chondrogenic differentiation, followed by hypertrophy and mineralization of the cartilage matrix. Subsequently, the cartilage is resorbed by osteoclasts and remolded into bone (Pierce and Perrien, 2021). The “seed cells” of HO differ across tissues, but the underlying mechanism remains similar. Retinoid signaling, mediated by nuclear retinoic acid receptors (RARs), including RARα, β, and γ, plays a crucial role in chondrogenesis and skeletal formation (Weston et al., 2003; Pacifici, 2018). This includes decreased retinoid signaling required in chondrogenic cell differentiation and cartilage maturation (Pignolo and Pacifici, 2021), providing new insights for treatment. Moreover, Yang et al. (2021) found that increased BMP signaling via caACVR1-induced cranial neural crest cells (CNCCs) in mice towards a chondrogenic fate by stimulating mTOR complex 1 (mTORC1) activity and subsequently suppressing autophagic degradation of β-catenin (Fig. 1). As mentioned earlier, the Ephrin B signaling pathway, identified in the EVs of FOP patients, could enhance endochondral ossification in fracture repair and may play a similar role in FOP (Hrkac et al., 2022).

4.1.3 FOP: a potential segmental progeroid syndrome

Pignolo et al. (2020) observed similarities between certain features related to dysregulated BMP signaling in FOP and those seen in accelerated aging, indicating

that FOP might be considered a segmental progeroid syndrome. They proposed that hypoxia, cellular damage, and inflammation resulting from soft tissue injury could lead to the accumulation of senescent cells, similar to what is observed in senescent tissues. They also posited that aging could promote tissue transformation towards cartilage formation in FOP muscle (Wang et al., 2022). This perspective provides valuable insights for potential therapeutic interventions in FOP.

4.1.4 Dysregulated temperature response in FOP

Wang et al. (2021) found that FOP CTPCs exhibited a dysregulated temperature response, and chondrogenesis was temperature-sensitive and was amplified in FOP CTPCs. This discovery adds temperature to the list of microenvironmental factors that likely influence FOP and suggests potential therapeutic avenues, such as antipyretics.

4.1.5 Decreased apoptosis levels in FOP

Stanley et al. (2022), building on previous studies of FAP, found that *Acvr1*$^{R206H/+}$ FAPs failed to decline due to significantly decreased levels of apoptosis during regeneration in FOP mouse models. This suggests that apoptosis-related modulators targeting FAP may be new therapeutic candidates.

4.2 Drug treatments

4.2.1 Retinoid agonists

4.2.1.1 NRX195183

Based on the result that all-*trans*-retinoic acid (atRA) treatment of limb mesenchymal skeletal precursor cells blocks chondrogenesis and cartilage formation, Shimono et al. (2010) tested the RARα agonist NRX195183 in an acquired HO mouse model with subcutaneous implantation of recombinant human BMP-2 (rhBMP-2)/Matrigel scaffolds, and found that NRX195183 reduced HO formation.

4.2.1.2 Palovarotene

Shimono et al. (2011) developed the RARγ agonist palovarotene (also known as R667) based on the usefulness of NRX195183. Palovarotene is a highly specific RARγ agonist, effectively inhibiting spontaneous HO in *ACVR1*^{R206H} mice (Chakkalakal et al., 2016) (Fig. 2). Several Phase II (NCT02190747, NCT02279095, and NCT02979769) and Phase III (NCT03312634) clinical trials have recently been completed, and some results are pending. The single-arm, open-label, Phase III MOVE trial (NCT03312634) showed that mean annualized new HO volume was 60% lower in the MOVE group than in the natrural history study (NHS) (Pignolo et al., 2023). Consequently, palovarotene (Sohonos) was recently approved by the FDA for reducing the volume of HO in adults and children aged eight years and older for females and ten years and older for males with FOP. However, it has been observed to cause premature maturation and closure of growth plates in some FOP patients (Pignolo and Pacifici, 2021), and there is an increased risk of vertebral fracture in patients treated with palovarotene (Pignolo et al., 2023). Other potential risks include pancreatitis, hearing and vision impairment, mouth ulcers, and dry skin (Kitoh, 2020).

4.2.2 Senolytic drugs: dasatinib and quercetin

Approaching FOP as a segmental progeroid syndrome, Wang et al. (2022) used dasatinib and quercetin to clear and reduce the senescence-associated secretory phenotype (SASP) (Fig. 2). The results showed that these senolytic drugs reduced BMP pathway signaling in FOP SHED and ameliorated HO in FOP mouse models.

4.2.3 PI3K inhibitor alpelisib

Phosphoinositide 3-kinase α (PI3Kα) is critical for bone formation through the regulation of SMAD1 activity (Gámez et al., 2016), and mTOR signaling, downstream of PI3K/protein kinase B (AKT), plays a critical role in HO and FOP (Hino et al., 2018). Valer et al. (2019) found that the PI3K inhibitor alpelisib (also known as BYL719 and marketed as Piqray™) was effective in suppressing HO in the *ACVR1*^{Q207D} mouse model. Alpelisib was well tolerated and was approved by the FDA for hyperactive PI3K phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)-altered solid tumors (Rugo et al., 2020), with the most frequent adverse effects being hyperglycaemia and rash (Ventura et al., 2021). PI3Kα inhibitors could negatively target ACVR1 kinase activity (Qureshi et al., 2017). However, further studies are required to optimize alpelisib treatment in FOP.

5 Discussion

Despite ongoing efforts, the explicit mechanism and ideal drugs for FOP remain elusive. Several

challenges remain, particularly concerning the specificity of ACVR1 inhibitors. Current inhibitors have a limited ability to distinguish between ACVR1 and ACVRL1, as well as between WT ACVR1 and FOP-related ACVR1, and their use would result in side effects.

As research into FOP deepens, new mechanisms of its occurrence and progression are being discovered, providing new therapeutic targets. However, the cell/tissue/stage specificity of new drugs remains a challenge, as evidenced by palovarotene causing premature maturation and closure of growth plates in some FOP patients. Furthermore, there are concerns regarding the targeting efficacy of FOP therapies that have shown initial clinical promise, necessitating subsequent modifications to enhance targeting and reduce side effects. Combining drugs with novel technologies, such as engineered exosomes, may ensure effective and precise targeting of therapeutic drugs to the tissues affected by FOP.

In addition, while many drugs have proven effective in animal models of FOP, the mechanisms of these drugs need further exploration. For instance, dasatinib, both a senolytic drug and an Src inhibitor, is effective in ameliorating HO by clearing and reducing SASP (Wang et al., 2022). Given that four Src kinase family members were identified in the EVs of an FOP flare-up group (Hrkac et al., 2022), dasatinib may also play a role in inhibiting HO by influencing this aspect.

The development of therapeutic drugs for FOP relies on progress in understanding the mechanisms underlying its occurrence and progression, as well as studies of its identification and confirmation of targets. Emerging technologies, such as genomics, proteomics, and high-throughput screening, can be used to identify potential targets, detect specific molecules or pathways that are dysregulated in the disease, offer new possibilities for old drugs, and validate them in vitro and in vivo to design new therapeutic drugs. Through a cellular NanoBRET target engagement assay, Fortin et al. (2020) identified a binding interaction between E6201 and ACVR1. E6201, an ATP-competitive mitogen-activated protein kinase kinase 1 (MEK1) inhibitor, has been reported to be effective in serine/threonine-protein kinase B-Raf (BRAF) V600E-mutated metastatic malignant melanoma (Babiker et al., 2019). It inhibits the activation of the BMP pathway induced by caACVR1 and shows a stronger inhibitory effect on pathway activation induced by mutant *ACVR1* compared with WT *ACVR1* (Fortin et al., 2020). Moreover, E6201 prolongs survival in mice xenografted with SU-DIPG-XXXVI or HSJD-DIPG-007 tumor cells carrying FOP-related *ACVR1* mutations (Fortin et al., 2020). Although the drug was not developed to target FOP, it could provide some clues for drug development.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) is also an emerging technology. Using an improved CRISPR/Cas9 system with high specificity, Kawamata et al. (2023) succeeded in editing a mono-allelic 617 G>A (R206H) *Acvr1* mutation in WT/WT murine embryonic stem cell (mESC), which subsequently causes ectopic ossification in mouse. This method greatly improves the efficiency of editing, but the effect of reverse application of this method to treatment remains to be further studied. However, given the regenerative capacity of progenitors/stem cells and the enhanced immune response in FOP, the cost performance of CRISPR/Cas9 for clinical treatment remains to be determined.

6 Conclusions

The treatment of FOP has long posed significant challenges for medical professionals due to the rarity and variability of the disease. However, years of continuous studies on FOP have led to a deeper understanding of its mechanisms, thereby guiding the development of new drug candidates aimed at alleviating patients' suffering. Ongoing research and clinical trials provide hope for improved management strategies and treatment options. Encouragingly, we are gradually progressing toward a potential "cure."

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Author contributions

Yijun ZHOU wrote and edited the manuscript. Ce SHI edited the manuscript. Hongchen SUN reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Yijun ZHOU, Ce SHI, and Hongchen SUN declare that they have no conflicts of interest.

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