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Common mutations in ALK2/ACVR1, a multi-faceted receptor, have roles in distinct pediatric musculoskeletal and neural orphan disorders

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Abstract

Activin receptor-like kinase-2 (ALK2), the product of *ACVR1*, is a member of the type I bone morphogenetic protein (BMP) receptors. ALK2 exerts key and non-redundant roles in numerous developmental processes, including the specification, growth and morphogenesis of endochondral skeletal elements. There is also strong evidence that BMP signaling plays important roles in determination, differentiation and function of neural cells and tissues. Here we focus on the intriguing discovery that common activating mutations in ALK2 occur in Fibrodysplasia Ossificans Progressiva (FOP) and Diffuse Intrinsic Pontine Gliomas (DIPGs), distinct pediatric disorders of significant severity that are associated with premature death. Pathogenesis and treatment remain elusive for both. We consider recent studies on the nature of the *ACVR1* mutations, possible modes of action and targets, and plausible therapeutic measures. Comparisons of the diverse – but genetically interrelated – pathologies of FOP and DIPG will continue to be of major mutual benefit with broad biomedical and clinical relevance.

Graphical Abstract

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Conflicts of interest

One of the authors (MP) is a consultant for Clementia Pharmaceuticals.

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Possible pathogenic mechanisms in FOP and DIPG

Keywords

ACVR1; ALK2; Fibrodysplasia Ossificans Progressiva; Diffuse Intrinsic Pontine Gliomas; Orphan diseases; BMP signaling

1. Introduction

ALK2, the product of *ACVR1*, is a member of the TGF β family subgroup of type I bone morphogenetic protein (BMP) receptors that includes ALK1, ALK3 and ALK6 (1). ALK2 partners with type II BMP receptors - including BMPRII and activin receptor type-2A (ACVRIIA) - to form tetrameric complexes that signal mainly via the canonical SMAD1/5/8 BMP signaling pathway in response to ligand binding but can also activate non-canonical signaling such as through p38MAPK (1, 2). ALK2 is able to bind several ligands in vitro such as BMP4, BMP6, BMP7, BMP9 and activins, but the identity and spectrum of its natural ligands in vivo are not clear (3–5). ALK2 exhibits the typical domain structure of ALK receptors that includes a ligand-binding extracellular domain, a transmembrane domain, a juxtamembrane glycine-serine-rich (GS) domain and the large kinase domain (6). Type I receptor kinases are activated by phosphorylation of their GS domain by the type II receptor kinases within the tetrameric complexes. Recent crystallography studies of ALK2 cytoplasmic region (7) have indicated that the kinase domain has a typical bilobal architecture and the GS domain extends from the N-lobe into a helix-loop-helix motif that can bind the endogenous inhibitor FKBP12 (8, 9). Those studies have additionally revealed that FKBP12 prevents access to the regulatory GS loop and inhibits α C movements needed for kinase activation and that the inactive conformation of the region is relatively stable even in the absence of FKBP12 (7), indicating that ALK2 conformation and activity are closely monitored and regulated through multiple mechanisms under normal circumstances.

Acvr1 is expressed in specific spatio-temporal patterns during mouse development (10) and its function appears to be required and non-compensable. Indeed, studies showed that global ablation of *Acvr1* arrests mouse embryogenesis at very early stages with a failure of the primitive streak to undergo gastrulation, produce mesoderm and extraembryonic tissues, and express critical mesoderm master genes such as *Brachyury* (11). Because of such early embryo lethality, conditional mutants were subsequently created to gain insights into ALK2

tissue- and organ-specific function over developmental and postnatal time. For example, *Acvr1* ablation in lens-forming head ectoderm via *Pax6-Cre* was found to lead to under-growth of lens primordia, abnormal regionalization of lens epithelium, poor expression of lens-characteristic genes, and increased apoptosis (12). *Acvr1* ablation in endocardial cushion mesenchyme caused aortic valve defects - including bicuspid aortic valve - that appear to result from substandard specification and differentiation of leaflet precursor cells and an altered balance between canonical (pSMAD1/5/8) BMP signaling and non-canonical p38MAPK signaling (13). By similar approaches, *Acvr1* was found to regulate other developmental processes including epithelial-mesenchymal transformation, Mullerian duct regression, and primordial germ cell specification (12, 14), firmly establishing ALK2 as a multi-faceted regulator of embryonic development and growth via direct or indirect action on multiple distinct cell types and tissues and events.

With regard to the musculoskeletal and neural systems that are the focus of this review, several studies have demonstrated that ALK2 regulates several aspects of skeletogenesis and skeletal growth, though less is known about its roles in neural development. Analysis of *Acvr1* ablation in neural crest cells via *Wnt1-Cre* indicated that ALK2 is required for specification and growth of several facial bones, cell proliferation in cartilage, and fusion of palatal shelves, though ALK2 did not appear to regulate neural crest cell migration and survival (15). *Acvr1* is strongly expressed in the resting and proliferative zones of the growth plates in developing and elongating skeletal elements, with lower expression in the maturing and hypertrophic zones (5), but the developmental significance and roles of these patterns had remained unclear. Recently, Rigueur et al. ablated *Acvr1* in developing mouse embryo cartilage via *Col2-Cre* (16), and found that although mutants were born at expected Mendelian ratios, they exhibited substandard growth of multiple endochondral skeletal elements including vertebral bodies and the cranial base, accompanied by a reduction in chondrocyte proliferation and lower levels of canonical and non-canonical BMP signaling. Compound *Col2-Cre*-driven *Acvr1/Bmpr1a* and *Acvr1/Bmpr1b* mutants were found to be perinatal lethal and exhibited severe chondrodysplasias (16). These interesting data indicate that ALK2 has a positive, non-redundant and encompassing role in cartilage development, growth and function and exerts coordinated activity with BMPR1A and BMPR1B during initiation, growth and morphogenesis of the endochondral skeleton.

Central and peripheral nervous system development and growth are processes of considerable complexity that involve multipotent progenitors, distinct cytodifferentiation pathways, local and systemic influences, and complex morphogenetic processes (17). Studies dating back over 2 decades have provided clear evidence that BMP ligands and BMP pathway signaling have multiple and dynamic roles in neural stem cell fate and differentiation and in overall CNS development and maintenance (18, 19). Inhibition of BMP pathway signaling is required for specification and formation of the neuroectoderm, while BMP signaling is needed to induce the neural crest (20–22). BMP signaling patterns and gradients establish the dorso-ventral axis of the spinal cord, influence specification and morphogenesis of forebrain and cerebellum and their native cell types, and regulate neuronal subtype identity and neurite outgrowth (23–25). Of particular relevance to this review are studies showing that *Acvr1* is expressed in many tissues in mouse embryos including the

cerebral hemispheres, medulla and thalamic regions, with little or no expression in the pons, a region that is rich in *Acvr1B* (*Alk4*) transcripts (10, 11, 26). Also relevant are subsequent studies on astrogliogenesis and neuronal cell lineage commitment (27). BMP signaling has been found to promote astroglial cell differentiation via a STAT3-p300/CBP-Smad1 complex cascade involving both canonical and non-canonical signaling pathways (28–30). Oligodendrogenesis and neurogenesis are promoted by basic helix-loop-helix (bHLH) transcription factors including Olig1, Olig2, Mash1, neurogenin and NeuroD (31). In neural precursors, BMP signaling has been found to stimulate expression of *Id1*, *Id3* and *Hes-5* that, by virtue of their ability to inhibit bHLH transcription factors, allow BMP signaling to stimulate astroglial commitment and differentiation while inhibiting neural cell differentiation (32).

This brief synopsis makes it clear that BMP proteins, receptors and signaling play important roles in musculoskeletal and brain development, growth, morphogenesis and postnatal function. It is not at all surprising then that aberrations in such key regulatory mechanisms cause developmental problems and pediatric disorders. In this review, we focus on Fibrodysplasia Ossificans Progressiva (FOP) and Diffuse Intrinsic Pontine Glioma (DIPG), distinct pediatric disorders of significant severity that are almost invariably associated with premature death. Intriguingly, many FOP and DIPG patients bear common activating mutations in *ACVR1*, raising questions about shared pathogenetic mechanisms of these diseases and the effects of, and responses to, common mutations in distinct cell types and populations.

2. Fibrodysplasia Ossificans Progressiva

FOP is a rare and extremely severe genetic musculoskeletal disorder with an incidence of about 1 in 2 million. The disease is characterized by episodic formation of extraskeletal bone tissue – a process termed heterotopic ossification (HO) – at multiple sites that accumulates over time and eventually involves much of the body (Fig. 1) (33). As a result, the accumulated ectopic tissue causes several health problems and impediments – including skeletal deformities, chronic pain, growth impairment and joint ankylosis – that increasingly interfere with basic daily functions and can lead to breathing difficulties and premature death (34). The HO process in FOP patients can be triggered by injury or small traumas, but is most often spontaneous and initiates with a local “flare-up” characterized by swelling, redness and pain (35). Because of the reactive nature of FOP, surgery to remove the HO lesions or correct skeletal defects is strictly avoided. In addition to excluding surgery as a treatment option, this also limits access to surgical retrieval specimens for diagnostic and prognostic purposes and pathogenesis studies. However, specimens have occasionally become available over the years either from misdiagnosed interventions or autopsies. Analysis of such samples has shown that formation of the heterotopic bone typically involves a recapitulation of the endochondral ossification process (36) by which most skeletal elements including long bones, ribs, vertebrae and cranial base form and grow during embryogenesis (37). As such, the process of HO formation involves the recruitment, condensation and proliferation of skeletal progenitor cells; their differentiation into chondrocytes; organization of growth plate-like structures permitting maturation and hypertrophy of the chondrocytes themselves; recruitment and invasion of blood vessels and

associated osteoprogenitors; and finally replacement of hypertrophic cartilage with endochondral bone (37). Longitudinal x-ray monitoring of the HO lesions suggests that the ectopic bone masses reach a given size and then stop growing, likely following the completion of the cartilage-to-bone transition and/or physically limited for example within the bounds of the fascia surrounding skeletal muscle.

2.1 ACVR1 genetic mutations and FOP clinical presentation

To date, a total of 13 heterozygous *ACVR1* missense mutations and one 3-bp deletion (eliminating two amino acids and inserting a different amino acid) at highly conserved positions have been described in FOP patients (Fig. 2) (38–41). About half of the mutations occur in the GS activating domain and the remainder occur in the protein kinase domain, with none reported so far in the transmembrane or extracellular ligand binding domains. Protein modeling and direct functional analyses have indicated that all the mutations are gain-of-function and slightly increase basal ALK2 signaling and downstream transcriptional activity (33, 35). However, the clinical presentation and consequences of these mutations vary, suggesting genotype-phenotype correlations and characterization of FOP patients in distinguishable subgroups (41).

Patients with a “classic” clinical presentation of FOP all bear the identical heterozygous mutation (c.617G > A; p.R206H) and share several clinical features including an early onset of HO before age 10 and malformation of great toes (hallux valgus), together with a frequent incidence of proximal tibial osteochondromas and cervical spine malformations (42). Within this group carrying the R206H mutation, phenotypic variability – such as in age of HO onset and rate of HO progression – has also been noted. Some patients with the R206H mutation and one patient with a Q207E mutation were reported to have additional clinical problems such as significant growth retardation, cataracts and aplastic anemia (41).

Even greater phenotypic variability is seen among patients who have non-R206H *ACVR1* mutations. For example, patients with the G328W or G328E mutation have very early onset HO, and instead of the characteristic specific great toe malformation, have reduction of multiple digits and thumb malformations, in addition to mild cognitive impairment and sparse scalp hair (41). Conversely a mild clinical phenotype was found in a patient with a G325A mutation who displayed the great toe malformation, but first developed HO at age 47 (43).

It remains unclear exactly how and why different mutations elicit distinct clinical presentations and trigger different pathological effects on skeletal and non-skeletal tissues and organs. Studies with very rare identical twins with FOP indicate that the environment affects the course of HO in a given patient and is a major influence on disease onset, progression and severity, in addition to the influences of genetic factors and the specific *ACVR1* mutation (44).

Because FOP is usually severe and limits reproductive capacity and life expectancy, most cases are sporadic and are thought to arise from germline mutations that would begin to exert their pathogenic effects starting from embryogenesis, as depicted by the great toe malformation visible at birth. However, it is also possible that some patients may have

acquired their ACVR1 mutation at a later embryonic or neonatal point and thus be mosaic; this could potentially be the case for patients without the characteristic skeletal malformations or with late-onset HO and/or very mild forms of the disease.

Protein crystallography and biochemical studies have examined the basis and mode of action of several of the FOP-associated ALK2 mutants, with particular attention given to the R206H mutation seen in classic FOP (7, 45–48). Plasmid-driven protein expression and reporter assays in cultured C2C12 cells for ten ALK2 mutants - including L196P, R206H, Q207E, G328E, P197_F198delinsL, G328R and R375P – were all found to display mildly-elevated basal activity in the absence of exogenous BMP treatment compared to wild type ALK2 (7). Such leaky activity was generally higher in GS mutants than kinase domain mutants. Interestingly, several mutants, including R206H and G328R, were still responsive to, and influenced by, their endogenous inhibitor FKBP12 as indicated by increased signaling activity after treatment with the FKBP12 antagonist FK506. One exception was the GS mutant L196P whose mildly-elevated basal activity was not further enhanced by FK506 treatment (7, 49), consistent with the very mild form of FOP exhibited by patients with this mutation (50). Structurally, the FOP mutations spatially center around the GS domain and the neighboring ATP pocket in the kinase domain (7, 47). The affected residues normally maintain the α -helical structure of the GS domain as well as critical bonds between the GS domain and the kinase N-lobe that are needed to stabilize the inactive conformation of ALK2, thus likely destabilize that conformation, altering its sensitivity to FKBP12 and eliciting an enhanced basal signaling activity. A partial loss of auto-inhibition had been initially proposed to explain the action of FOP ALK2 mutants (41, 48) and reduced binding of mutant ACVR1 to FKBP12 has been experimentally supported (51).

Given the nature of the mutations, it was of interest to ask whether the gain-of-function ALK2 mutants still require cooperation with BMP type II receptors and/or phosphorylation of their GS domain by the type II receptors to express their leaky activity, as is required by wild type ALK2. To approach these questions, Bagarova et al. (43) expressed plasmid-driven wild type ALK2, ALK2^{R206H} or ALK2^{Q207D}, the latter being an engineered constitutive-active mutant (52), in wild type mouse cells and in cells deficient in *BmpRII* and/or *ActRIIa*. Reporter assays showed that both GS mutants increased basal signaling activity in both wild type cells and those deficient in either type II receptor, but not in cells deficient in both receptors, supporting the idea that ALK2 mutants require cooperation with type II receptors for activity. Interestingly, however, the ALK2 mutants did not appear to require type II receptor kinase activity nor ligand binding activity to elicit their enhanced basal action, providing further evidence that the GS mutations destabilize the inactive configuration of ALK2 and render it leaky, independently of ligand binding and phosphorylation.

2.2 ACVR1 genetic mutations and endochondral bone formation

As noted above, the HO process in FOP closely mimics endochondral ossification in which progenitor cells undergo chondrogenesis and cartilage formation, become organized into growth plates and are then replaced by bone and marrow (36). Thus, it is pertinent to ask whether the ALK2 mutants have enhanced chondrogenic and osteogenic capacity and

whether an ALK2 mutation is sufficient to cause HO and FOP. A first study addressing these key questions was carried out with the constitutive-active *ALK2*^{Q207D} mutant (52) using a virally-driven over-expression approach in chick embryo limbs (5). *ALK2*^{Q207D} over-expression was found to cause significant over-growth and lateral expansion of the developing cartilaginous elements and led also to fusion of synovial joints, a process previously seen in mutants such as *Noggin*-null mouse embryos which form excess cartilage at the prospective location of the joints (53). At the cellular level, *ALK2*^{Q207D} over-expression increased the rate of chondrocyte maturation and hypertrophy and caused higher levels of expression of the key growth plate signaling protein Indian hedgehog (54), indicating again that cartilage cells are quite sensitive and respond positively and exuberantly to mutant ALK2 action. In line with these results, van Dinther et al. found that plasmid-driven over-expression of the FOP mutant *ALK2*^{R206H} in COS cells not only enhanced basal BMP/SMAD reporter activity, but also rendered the cells more sensitive to osteogenic cell differentiation and mineralization in response to exogenous BMP treatment compared to cells over-expressing wild type ALK2 (55). They also found that human mesenchymal stem cells that over-express *ALK2*^{R206H} and are implanted subcutaneously in nude mice produced more ectopic bone than cells expressing control ALK2. In good agreement, one of us reported recently that *Alk2*^{R206H} expression in mouse embryo fibroblasts enhanced their chondrogenic differentiation and responsiveness to BMP treatment; when implanted in vivo, the *Alk2*^{R206H/+} cells initiated robust HO and stimulated recruitment and participation of surrounding wild-type cells in the process (56). Interestingly, additional data in the study showed that ablation of *Alk2* at the early commitment stage of differentiation compromised the ability of the progenitor cells to undergo chondrogenesis, reiterating *Alk2*'s importance and non-redundant function.

Though revealing and instructing, in vitro studies cannot address the related question whether an FOP mutation is sufficient to elicit the FOP phenotype. Accordingly, knock-in mice with the classic R206H mutation inserted in the *Acvr1* locus were developed by one of us recently and were extensively phenotyped for FOP-associated features (57). Radiographic and anatomical analyses of founder generation chimeric *Acvr1*^{R206H/+} mice showed that the mutation induced malformation of the first digits in the hind limbs and postnatal extraskelatal bone formation, thus closely recapitulating the key characteristic features of classic FOP. Further histological and molecular analyses showed that the HO process was preceded by local inflammatory cell infiltration, apoptosis of skeletal muscle cells, a robust fibroproliferative response, followed by endochondral ossification, and that cells with a Tie2+ lineage (a marker of endothelial cells and muscle interstitial progenitor cells) participated in the formation of extraskelatal tissue. In addition to extensive spontaneous HO formation, this mouse model also formed HO in response to injury. The study thus provided clear evidence that the classic R206H mutation is sufficient to cause an overall phenotype in mice that very closely resembles FOP, and mimics the pathogenic steps and phases leading to HO. These data and conclusions agree with other studies showing that the engineered constitutive-active Q207D mutation can also provoke robust and even severe HO in mice expressing a conditionally-inducible *ACVRI*^{Q207D} transgene (52, 58, 59).

Taken together, these studies have provided strong support for the notion that ALK2 normally acts as an important and direct regulator of commitment of skeletogenic progenitors and their differentiation into cartilage and bone. Its mutant and activated forms can, and do, favor and propel these processes, thus playing an instigating role in HO and a causative and sufficient role in FOP.

2.3 FOP current and potential treatments

Almost invariably, HO in FOP patients is preceded by local inflammation in the form of a spontaneous flare-up or following tissue injury and trauma that involves swelling, pain, erythema, stiffness and accumulation of mast cells and other immune cells (33). Because such an initial inflammatory phase is likely to set the stage for, and instigate the onset of, HO, the current standard of care for FOP patients is a brief 4-day course of high-dose corticosteroids (60). Treatment is normally started within the first 24 hours of a flare-up and aims to reduce the inflammation and edema and in turn, the likelihood of progression to HO. In an analogous manner, non-steroidal anti-inflammatory drugs (NSAIDs) are also considered an option as maintenance medications for FOP patients (60). These drugs inhibit the synthesis of both physiologic and inflammatory prostaglandins (61), and animals pretreated with prostaglandin synthesis inhibitors were originally shown to be resistant to experimental HO induced by intramuscular injection of BMPs (62). Though the steroid and non-steroid anti-inflammatory treatments can and do mitigate inflammation, swelling and pain, they have not been found to consistently reduce the frequency of progression to HO in FOP patients.

The extracellular matrix in musculoskeletal tissues contains and stores a large amount of growth factors, and many studies have indicated that the release of these factors by the action of osteoclasts stimulates osteoblast function and couples bone resorption to bone formation (63, 64). This line of evidence and other considerations have led to the indication that aminobisphosphonates could represent a possible treatment for FOP (60, 65, 66). In addition to interfering with osteoclast function and survival, and in turn bone formation, this class of drugs also has strong anti-angiogenic effects in the context of cancer (67). Because angiogenesis is required during the transition from hypertrophic cartilage to bone (68), the aminobisphosphonates could benefit FOP by blocking this needed developmental process during a similar transition that occurs within the heterotopic cartilaginous-bony tissue mass. Experimental studies showed that systemic treatment with bisphosphonates inhibits HO in trauma- and BMP4-induced mouse models (69). These studies indicated also that the drug treatment reduced presence and activities of monocytes within the lesion area, and there is long standing evidence that monocytes, mast cells and other immune cells may be an integral part of local mechanisms by which HO is instigated and initiated in FOP (70, 71). Potentially then, aminobisphosphonates could interfere with HO at a variety of regulatory levels. Despite these compelling findings and studies, however, the extent to which the drugs represent an effective and safe treatment for FOP remains unclear, even when used in combination therapy to protect the skeleton from the osteopenic effects of high-dose glucocorticoids (72).

The discovery of activating *ACVRI* mutations in FOP patients (48) has since propelled research into how such aberrant activity could be blocked or at least reduced, thus counteracting what is undoubtedly the key regulatory pathogenic change in the disease. Conceivably, this could be achieved by blocking the natural BMP ligands for ALK2 or additional ligands possibly utilized by mutant ALK2, interfering with the leaky signaling activity of mutant ALK2 by either suppressing its kinase activity or restoring its normal configuration and interactions via specifically-designed drugs, or counteracting the differentiation steps of progenitor cells recruited to the prospective HO site. In line with the second possibility, Yu et al. reported compelling findings that the selective inhibitor of BMP type I receptor kinases LDN-193189 significantly reduced HO in the limbs of transgenic mice carrying an inducible and constitutive-active *ACVRI*^{Q207D} transgene (59).

LDN-193189 is a compound optimized for pharmacokinetic characteristics and stability (73) and is a modified version of Dorsomorphin, a previously identified inhibitor of ALK2, ALK3 and ALK6 and their canonical SMAD1/5/8-mediated signaling activity (74). Yu et al. found that the drug treatment reduced phosphorylation and activation of SMAD1/5/8 in the affected tissues expressing *ACVRI*^{Q207D} and also reduced functional impairment and maintained limb function. In follow-up studies, this group and collaborators developed a more potent inhibitor of the type I receptor kinases – LDN-212854 – that displayed a nearly 4-fold higher selectivity for BMP versus related Activin and TGF- β type I receptors compared to previous kinase inhibitors (75, 76). They showed that LDN-212854 exhibited some selectivity for ALK2 compared to ALK1 and ALK3 in cultured cells and as importantly, inhibited HO in the inducible *ACVRI*^{Q207D} transgenic mouse model. These studies are quite promising and exciting and point to the possibility that type I receptor kinase inhibitors with the necessary specificity and selectivity can be created to be effective against HO and with the appropriate safety profile in FOP patients.

To create a strategy to interfere with cellular processes and differentiation during HO, one of us and collaborators focused on the early developmental steps of HO in which the progenitor cells recruited to the putative HO site are induced to undergo chondrogenesis and cartilage formation by the combined action of mutant ALK2 and other as yet unknown mechanisms (36). We focused on the chondrogenic phase because it is an obligatory early step in endochondral ossification (37) that if blocked, would in turn prevent the subsequent formation of bone and thus halt the overall HO process on its tracks. There are several experimental ways by which chondrogenesis could be inhibited, including genetic and pharmacologic means (37). We targeted the retinoid signaling pathway and its nuclear retinoic acid receptors (RAR α , RAR β and RAR γ) (77) for the following reasons. Biological and genetic studies had previously demonstrated that chondrogenic cell differentiation in vitro and chondrogenesis in vivo normally require a steep decrease in both endogenous retinoid signaling and RAR expression (78, 79), and that exogenous retinoid agonists are very potent inhibitors of chondrogenesis (80). In two recent studies, we found that synthetic retinoid agonists for RAR α and RAR γ given systemically by oral administration or injection were effective inhibitors of HO in mouse subdermal and intramuscular injury models and the genetic *ACVRI*^{Q207D}-inducible transgenic mouse model, with the RAR γ agonists being far more effective (58, 81). In vivo and in vitro evidence showed that the retinoid agonists had blocked the chondrogenic phase of HO and showed no significant rebound effect over

time after end of treatment. Mechanistically, the agonists appeared to act by not only blocking expression of master chondrogenic genes such as *Sox9*, but also greatly diminished canonical pSMAD1/5/8-mediated BMP signaling and promoted SMAD degradation via the proteasome (58). The latter data fit well with previous evidence that there is an antithetical relationship between retinoid signaling and BMP signaling during chondrogenesis and that the normal pro-chondrogenic roles of BMPs in cartilage development and skeletogenesis depend on the attenuation of endogenous retinoid signaling (82, 83). In related studies, we found that retinoid agonists increase the activity of the Wnt/ β -catenin signaling pathway (84) which is known for its strong anti-chondrogenic action (85, 86), implying that the retinoid agonists block chondrogenesis and HO by concurrently inhibiting BMP signaling and stimulating Wnt/ β -catenin signaling. One of the most effective RAR γ agonists was Palovarotene, a drug that was previously tested in a phase 2 clinical trial for a different chronic condition and found to be safe (87). Based on our studies, this repurposed drug is now being tested in a FDA-approved phase 2 clinical trial with FOP patients that was initiated by Clementia Pharmaceuticals in July 2014 (ClinicalTrials.gov identifier NCT02190747). The trial is expected to last 2 years and given its double-blind and placebo design, should provide definitive evidence as to whether Palovarotene is an effective and safe remedy against HO in FOP.

Interestingly also, a just published study has reported evidence of a new aspect of the pathogenesis of HO in FOP (88). Using in vitro systems and a conditional knock-in *ACVR1^{R206H}* FOP mouse model, the authors found that cells expressing mutant *ALK2^{R206H}* responded to activin A, a normally antagonistic ligand for wild type ALK2, and signaled via canonical pSMAD1/5/8. Treatment of FOP mice with neutralizing antibodies to activin A reduced the extent of HO formation. Although it remains to be established whether activin A antibodies could stop HO in FOP patients and do so safely, the study is exciting and establishes a concrete link between inflammation and HO, given that activins are often produced by inflammatory cells (89). Note that since Palovarotene and other retinoid agonists block canonical BMP/SMAD1/5/8 signaling (58, 82), Palovarotene is expected to inhibit HO also by blocking BMP signaling induced by activin A via mutant ALK2, supporting its encompassing potency regardless of the nature of HO-inciting stimuli.

3. Diffuse Intrinsic Pontine Glioma (DIPG)

Brain tumors are the most common form of solid tumors affecting children and amount to about 20% of all pediatric cancers (90). The tumors can develop at any site within the brain, have diverse histopathological characteristics and phenotypes, and can remain benign or transition to malignancy over time (91). Brainstem gliomas represent a specific subgroup of brain tumors. About 15 to 20% of them are low-grade astrocytomas that include dorsally exophytic, cervicomedullary and focal brain gliomas, are characterized by specific biological and cellular traits including relatively slow cell growth, and often follow an indolent course with favorable survival for years or even decades (91, 92). The remaining 80 to 85% of brainstem gliomas are diffuse, occupy the ventral pons, have a peak age in middle childhood (6–7 years) and are currently referred to as diffuse intrinsic pontine gliomas (DIPGs) (93). Their histological classification as WHO grade IV reflects the fact that their cells display pleomorphic cytoarchitecture and structure, high density and nuclear atypia that

are associated with high mitotic activity in conjunction with microvascular proliferation (94). The tissue also can display areas of necrosis. DIPG occurs at an incidence of approximately 1 in a million with a very poor prognosis, leading to death within 1 to 2 years from diagnosis; DIPG is the main cause of death among all pediatric brain tumor patients (91). The understanding of DIPG biology and pathogenesis has been limited in the past due to the paucity of tumor tissue since biopsies were avoided due to the forbidding anatomical location of the tumors, along with morbidity and mortality concerns; however, the specific peak age of the patients and the specific anatomical location of the tumors hinted to abnormalities in postnatal neuro-developmental mechanisms (95). More recently, biopsy procedures that pose little to no concern have been developed, and rapid retrieval of autopsy material is an effective method to obtain DNA and RNA samples and cells to establish primary cultures and cell lines (96–98). Autopsy samples could harbor additional genetic changes due to exposure to radiation and/or chemotherapy by the patients that would not be present in biopsy samples (99). Nonetheless, these complementary technical, organizational and regulatory changes have paved the way to the recent major advances in understanding on DIPG biology and pathophysiology and to testing functionally defined therapeutics.

3.1 Diverse genetic aberrations in DIPGs, including ACVR1 mutations

A number of studies have demonstrated that DIPGs are characterized by gross chromosomal aberrations and imbalance, including gains at chromosomes 2, 8q and 9q, and by focal gene amplifications and deletions. Amongst the most common amplifications are those affecting *PDGFRA* and the receptor tyrosine kinases *MET*, *IGF1R* and *ERBB4* some of which occur simultaneously within the same tumor (99–101). Focal amplifications are also seen in genes involved in the PI3K signaling pathway, including *HRAS*, *AKT1* and *PIK3CA*, and genes within the retinoblastoma (Rb) pathway such as *CCND1*, *CCND2* and *CDK4*, with an overall frequency of about 40% in each cohort of patients. Deletions in the tumor suppressors *CDKN1C* and *CDKN2A/2B* have been observed in some cases (99–101).

Genome sequencing analyses of biopsy and autopsy samples more closely examined the genomic landscape of DIPG. Somatic heterozygous mutations in histone H3 isoforms, which characterize pediatric high grade gliomas (pHGGs), were found to be more frequent in DIPG tumors, with the specific missense K27M mutation in *H3F3A* seen in about 60% of the cases (102, 103). Mutations in the same codon were also found in the histone H3.1 gene (*HIST1H3B*) with a frequency of about 20% of cases. Although comparisons of different cohorts of pHGG and DIPG patients revealed some slight differences in the frequency of *H3F3A* versus *HIST1H3B* mutations, the H3.1 mutations were more characteristic of younger children and were largely restricted to DIPG (102–104). Both H3.3 and H3.1 mutations are thought to have a dominant-negative effect on the total histone H3 pool and lead to DNA hypomethylation by inhibition of the H3K27 methylase EZH2 and broad changes in gene expression (105).

Recent whole genome, whole-exome and transcriptome comparisons revealed that, in addition to somatic histone H3 mutations, other genetic changes are characteristically found in 50–60% of DIPG tumors including structural variants generating fusion genes, as well as mutations in genes involved in histone modification, chromatin remodeling and cell cycle

regulation (106). Mutations in the tumor suppressor protein TP53 are also very common in DIPG with an incidence of 40 to 50% of cases (107).

Of particular relevance here are four recent independent reports that collectively examined over 200 DIPG patients and showed that recurrent heterozygous somatic non-synonymous mis-sense mutations in *ACVR1* occurred in about 33% of DIPGs (70/209), but were absent in non-brainstem high-grade gliomas (Fig. 2) (106, 108–110). Seven amino acid substitutions were identified: R206H and Q207E in the glycine-serine-rich (GS) domain: and R258G, G328E, G328V, G328W and G356D in the protein kinase domain (Fig. 2). With the exception of G328V, all these mutations are identical to *ACVR1* mutation identified in FOP patients (Fig. 2). Of note, the *ACVR1*^{R206H} mutation occurs in more than 95% of FOP patients but is more rare in DIPG tumors, while the other *ACVR1* variant mutations, often associated with more severe forms of FOP, are more common in DIPG tumors.

In the study by Wu et al. (106), the six *ACVR1* mutations examined were found to be significantly associated with younger patient age, longer patient survival time, and presence of K27M H3.1 (*HIST1H3B*) mutation or *PIK3CA* or *PIK3R1* mutations, indicating that they may characterize a distinct subset of DIPG patients. As described above for FOP *ACVR1* mutations, the affected residues in the mutant DIPG *ACVR1* proteins normally maintain the α -helical structure of the GS domain and critical bonds between GS domain and kinase N-lobe that are needed to stabilize the inactive conformation of ALK2. Each *ACVR1* mutant was found to increase basal canonical BMP pathway signaling (pSMAD1/5) when over-expressed in mouse primary astrocytes in culture. Because a similar response is seen in skeletal cells (7, 45, 46), the effects of *ACVR1* gain-of-function mutants are clearly dominant with regard to signaling and not cell type-dependent, at least for mesenchymal and neural cells. However, the *ACVR1* mutants alone failed to impose a tumorigenic phenotype on *Tp53*-null mouse astrocytes when transplanted in the brain of host mice, indicating that the mutations are not tumorigenic *per se* but would exert roles in concert with other DIPG mutations.

In the study by Buczkowicz et al. (108), two of the *ACVR1* mutants tested (R206H and G328V) elicited increased proliferation when over-expressed in brainstem cell progenitors isolated from *Nes-tv-a;Tp53^{fl}* mice. Interestingly, while the *ACVR1* mutants induced pSMAD1/5 phosphorylation and expression of BMP pathway target genes *ID1* and *ID2*, the latter genes were also induced by over-expression of K27K H3.3 mutant and even more so by K27K H3.3 plus *ACVR1* mutant co-expression, suggesting possible additive effects and gene interactions in regulating cell behavior, differentiation and tumorigenesis.

Taylor et al. (110) found that the *ACVR1* mutations co-segregated with the histone H3.1 mutant, were twice as frequent in female patients, and correlated with longer overall patient survival times. The authors prepared DIPG case-derived primary cultures (two of which harbor *ACVR1* mutations) and found that treatment with the selective ALK2 antagonist LDN-193189 (59) caused marked decreases in both pSMAD1/5-mediated BMP signaling and cell viability in all cultures, indicating that the *ACVR1* mutations did not provide any major cell survival advantage at least within such experimental context.

Lastly, Fontebasso et al. (109) also observed an association of *ACVR1* mutations with *H3.1* rather than *H3.3* K27 M mutation and detected a strong increase in pSMAD1/5/8 immunostaining in DIPG brain samples compared to controls. They also found that primary DIPG cells carrying *H3.1* K27M and *ACVR1* G328V mutations exhibited higher pSMAD1/5/8 levels and higher *ID1*, *ID2*, *ID3* and *SNAIL* gene expression than cells with *H3.3* alteration and wild type *ACVR1*.

3.2 Significance and possible consequences of *ACVR1* mutations

While mutations in *ACVR1* are necessary and sufficient to cause FOP, it appears that the somatic *ACVR1* mutations are neither necessary nor sufficient in DIPG, although their high incidence undoubtedly points to possible major roles. As indicated and discussed in the recent DIPG genomic studies (106, 108–110), the *ACVR1* mutants must be part of far more complex pathogenic mechanisms and genetic interactions that eventually trigger abnormal cell behavior and elicit tumor initiation and growth (see schematic in Fig. 3). Based on the biology of *ACVR1* and mechanisms known to regulate its function in various developmental processes including brain development, several possibilities can be considered.

The mouse embryo studies cited above indicate that *Acvr1* expression normally characterizes the developing brain hemispheres and other brain structures but not the pons, indicating that aberrant *ACVR1* expression in the pons of DIPG patients in conjunction with activating mutations could derange the behavior of pons cells, altering their phenotype – for example via expression of ID genes and broad genome demethylation - and ultimately contributing to their malignant pleiotropic appearance and behavior. The pons normally expresses *ACVR1B* (*ALK4*), a type I receptor usually associated with TGFβ pathway (pSMAD2/3) signaling (111). If such expression were maintained in DIPG, the combination of mutant *ACVR1* plus endogenous *ACVR1B* could alter signaling and shift the balance between BMP and TGFβ signaling. A precedent for this possibility is found in the case of *ALK5* which normally signals via pSmad2/3 but can signal via pSmad1/5 in association with *ALK1* (112). BMP pathway signaling normally promotes astroglial cell differentiation via a STAT3-p300/CBP-Smad1 complex cascade involving both canonical and non-canonical signaling pathways (28–30) and thus, an imbalance of those and related pathways could derange the differentiation or differentiated status of local cells and contribute to growth and neoplastic behavior (113).

Genetic cell tracing and tracking have recently become a very popular tool to decipher the origin of progenitor cells and their roles in the formation of distinct tissues and organs during embryogenesis, the mechanisms regulating such complex cell developmental decisions and lineage assignment, and possible aberrations in these fundamental processes in pathologies including FOP (114–117). These powerful approaches have not been used extensively in DIPG research so far, but one notable exception is the study by Monje et al. (118). These authors first analyzed the midbrain, pons and medulla of postnatal human brains from age 0.3 to 18 years and found that immunodetectable Nestin+ progenitor cells (119) were specifically located in the pons in infancy, peaked in number around age 6, but dwindled with time. About 50% of pons-associated Nestin+ cells also expressed the HLH transcription factor *Oligo2* which is involved in divergence of neural progenitor

differentiation paths (31). Similar Nestin+/Oligo2+ cells were seen in the ventral pons region in young mice, and the cells exhibited also strong expression of *Sox2*, a marker of progenitor cells (120), and strong signaling by Sonic hedgehog which is known to regulate *Oligo2* expression during neural development (121). Ectopic hedgehog signaling led to a burst of Oligo+ progenitor cell proliferation in the pons, leading the authors to propose that these neural progenitor-like cells may represent a “cancer stem cell population” in DIPG and that hedgehog signaling could be an important (though not sufficient) pathogenic driver as well as a therapeutic target. Numerous studies have shown that the hedgehog signaling pathway works in close concert with the BMP signaling pathway to regulate numerous and major developmental processes and events (122). For instance, *Acvr1* induces and regulates expression of *Indian hedgehog* which in turn controls chondrocyte proliferation and maturation during skeletal development (5). Hedgehog protein gradients have been found to differentially interact with BMP signaling to regulate expression of the homeobox gene *engrailed2a* and the balance between proliferation and differentiation in myotomal progenitor cells (123). Thus, it is possible that aberrant expression of mutant *ACVR1* in DIPG could interact with basal - or in some cases upregulated (124) - hedgehog signaling and contribute to maintaining cells located in the most ventral pons region in a proliferative state, contributing to propagation of their stem/progenitor phenotype and ultimately sustaining tumor growth. Such local and niche-restricted actions and interactions have been observed in other systems as exemplified by Sonic hedgehog-mediated induction of proliferation of primitive hematopoietic cells via BMP signaling (125).

In a similar vein, it is well established that the BMP pathway exerts positive influences on the expression of *HOX* genes in a variety of developmental systems and processes. For example, dorsal BMP expression and signaling regulate *Hox* gene expression in the mammalian developing neural tube (126), and BMP action initiates *Hox* expression during early *Xenopus* embryogenesis (127). Interestingly, several *HOX* genes are up-regulated in both DIPG and pHGG, including *HOXA9*, *HOXA10*, *HOXA2* and *HOXA3* (128–130). *HOX* expression has been suggested to be linked to mutant histone H3-dependent and PI3K-dependent DNA de-methylation, to elicit a glioma stem-like cell phenotype on local cells, and exert anti-apoptotic and pro-proliferative effects (100, 130). Given the usually positive influence of BMP signaling on *HOX* expression, it is possible that ectopic mutant *ACVR1* expression in DIPG may reinforce these aberrant *HOX* expression patterns and contribute to sustain tumor growth and/or tumor stem-like cell phenotype.

3.3 DIPG therapeutic remedies

Recent comprehensive reviews have provided details and insights on, and critical assessment of, previous and novel therapeutic strategies that are being tested to treat DIPG and improve the currently dismal patient survival rates (91, 93, 100). Radiotherapy has long been used to treat adult HGG with considerable survival success and, in the absence of other options, has become the standard therapy for pHGG and DIPG with, unfortunately, limited success (91). Effectiveness of chemotherapy and combination chemotherapy have also been studied in all these disorders (131). Novel strategies are attempting to exploit the molecular information stemming from the genomic analyses described above. Accordingly, drugs are being tested singly or in combination with other drugs/irradiation to reverse DNA

hypomethylation (a major example being Temozolomide), block receptors including IGF1R, PDGFR and VEGFR, or interfere with signaling pathways such as PI3K/mTOR, Ras/Raf/MEK and CDK (132). Antibody and cell-based therapies are being studied as well. To identify functionally defined therapeutic drugs, a very recent and exciting study screened chemical drug libraries using patient-derived DIPG cell cultures and combined this approach with RNA-seq analysis and informatics and computational modeling (133). This comprehensive study led to the identification of the multi-histone deacetylase inhibitor Panobinostat. The drug markedly reduced viability and proliferation of DIPG cells in vitro, and inhibited growth of DIPG orthotopic xenografts implanted into the skull of host mice and extended mouse survival, particularly in combination with the histone demethylase inhibitor GSK-J4. The recent identification of *ACVR1* activating mutations in DIPG has led to the suggestion that interference with BMP signaling could represent an additional therapeutic target and provide for an even more comprehensive and effective combination therapy (106, 108–110).

The task of finding an effective treatment is daunting given the sheer complexity and multiplicity of the genetic alterations in DIPG. The lack of a faithful mouse models is certainly a major hurdle in deciphering the exact pathogenesis and natural history of DIPGs. In particular, what is needed is a better understanding of the temporal and spatial occurrence and sequence of genetic alterations and most importantly, what hierarchical position each alteration occupies with respect to the overall gene mutation landscape and to tissue and local cell pathogenic behavior. Based on differences in genomic landscape within one of the studies above, DIPG patients were suggested to represent three separate molecular subgroups (108). This is certainly possible, but it may also be that these subgroups represent steps along a pathogenic continuum and may have possessed, or would acquire, different genomic traits and signature over time. In this regard, the suggestion noted above that inhibition of excess signaling by mutant *ACVR1* could benefit DIPG is understandable, but what is not known yet is exactly what mutant *ACVR1* actually does in DIPG and whether its function changes with time, affects different subpopulations differently, and/or is affected by the specific genomic landscape at any given time. It is tantalizing that two of the above four studies actually reported longer survival times of DIPG patients bearing *ACVR1* mutations (106, 110). This makes one wonder whether these mutations are indeed pathogenic or may actually have or acquire some protective value.

4. Conclusions and perspectives

FOP and DIPG are diametrically distinct diseases and yet, they share the unfortunate facts that they are not currently preventable, are fatal, have a similar incidence, and become evident in early infancy. FOP children do not have DIPG although neurological symptoms have been observed in some FOP patients including allodynia, neuropathic pain, myoclonus and mild cognitive impairment (134, 135), and a recent report has described an FOP patient with abnormal soft tissue surrounding the brainstem leading to obstructive hydrocephalus and bilateral dentate lesions (136). Likewise, DIPG patients do not have FOP or obvious manifestations of heterotopic ossification. Germline *ACVR1* mutations during early development drive skeletal and developmental features of FOP and embryonic and post-natal expression in mesenchymal tissues lead to HO, whereas somatic and local mutations in

ACVRI add to the multiple genomic alterations and mutation burden in DIPG pathogenesis. As pointed out above, potent and potentially effective drug treatments for FOP have recently been discovered using genetic and injury mouse models of the disease (58, 74, 88), and we ourselves are engaged in the FDA-approved phase 2 clinical trial mentioned above to test the efficacy of the retinoic acid receptor γ agonist Palovarotene in FOP. A main mechanism by which Palovarotene blocks extraskelatal endochondral ossification is by potent inhibition of BMP signaling pathway (58). Thus, should *ACVRI* mutations be established as pathogenic in DIPG, one could certainly consider testing Palovarotene to counter the excessive BMP signaling in DIPG. On the other hand, *ACVRI* mutations may be determined to be protective in DIPG; for instance, rather than promoting stem cell renewal and tumorigenesis together with *HOX* genes in the ventral pons, the enhanced BMP signaling from mutant *ACVRI* could promote astroglial differentiation and mitotic quiescence (28–30). In such case, retinoid antagonists could be used (137) to boost BMP signaling (82). It is certain then that continuous efforts to decipher and understand the cellular and molecular pathogenesis of DIPG and FOP will be of significant and mutually beneficial value and critical basic, biomedical and clinical relevance, ultimately providing renewed hope to patients and families alike that effective treatments for these exceedingly severe conditions can be, and are being, developed.

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References

- Schmierer B, Hill CS. TGF β -SMAD signal transduction: molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol*. 2007; 8:970–82. [PubMed: 18000526]
- Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth Factors*. 2004; 22:233–41. [PubMed: 15621726]
- Clarke TR, Hoshiya Y, Yi SE, Liu X, Lyons KM, Donahoe PK. Mullerian inhibiting substance signaling uses a bone morphogenetic protein (BMP)-like pathway mediated by ALK2 and induces SMAD6 expression. *Mol Endocrinology*. 2001; 15:946–59.
- Luo J, Tang M, Huang J, et al. TGF β /BMP type I receptors ALK1 and ALK2 are essential for BMP9-induced osteogenic signaling in mesenchymal stem cells. *J Biol Chem*. 2010; 285:29588–98. [PubMed: 20628059]
- Zhang D, Schwarz EM, Rosier RN, Zuscik MJ, Puzas JE, O’Keefe RJ. ALK2 functions as a BMP type I receptor and induces Indian hedgehog in chondrocytes during skeletal development. *J Bone Min Res*. 2003; 18:1593–604.
- Huse M, Chen Y-G, Massague J, Kuriyan J. Crystal structure of the cytoplasmic domain of the type I TGF β receptor in complex with FKBP12. *Cell*. 1999; 96:425–36. [PubMed: 10025408]
- Chaikuad A, Alfano I, Kerr G, et al. Structure of the bone morphogenetic protein receptor ALK2 and implications for Fibrodysplasia Ossificans Progressiva. *J Biol Chem*. 2012; 287:36990–8. [PubMed: 22977237]

8. Huse M, Muir TW, Xu L, Chen YG, Kuriyan J, Massague J. The TGF β receptor activation process. An inhibitor-to-substrate binding switch. *Mol Cell*. 2001; 8:671–82. [PubMed: 11583628]
9. Wang T, Li BY, Danielson PD, et al. The immunophilin FKBP12 functions as a common inhibitor of the TGF- β family type I receptors. *Cell*. 1996; 86:435–44. [PubMed: 8756725]
10. Verschuere K, Dewulf N, Goumans MJ, et al. Expression of type I and type IB receptors for activin in midgestation mouse embryos suggests distinct functions in organogenesis. *Mech Dev*. 1995; 52:109–23. [PubMed: 7577669]
11. Mishina Y, Crombie R, Bradley A, Behringer RR. Multiple roles for activin-like kinase-2 signaling during mouse embryogenesis. *Dev Biol*. 1999; 213:314–26. [PubMed: 10479450]
12. Rajagopal R, Dattilo LK, Kaartinen V, et al. Functions of the type 1 BMP receptor Acvr1 (Alk2) in lens development: cell proliferation, terminal differentiation, and survival. *Invest Ophthalmol Visual Sci*. 2008; 49:4953–60.
13. Thomas PS, Sridurongrit S, Ruiz-Lozano P, Kaartinen V. Deficient signaling via Alk2 (Acvr1) leads to bicuspid aortic valve development. *PLoS ONE*. 2012; 7:e35539. [PubMed: 22536403]
14. de Sousa Lopes SM, Roelen BA, Monteiro RM, et al. BMP signaling mediated by ALK2 in the visceral endoderm is necessary for the generation of primordial germ cells in the mouse embryo. *Genes & Dev*. 2004; 18:1838–49. [PubMed: 15289457]
15. Dudas M, Sridurongrit S, Nagy A, Okazaki K, Kaartinen V. Craniofacial defects in mice lacking BMP type I receptor Alk2 in neural crest cells. *Mech Dev*. 2004; 121:173–82. [PubMed: 15037318]
16. Rigueur D, Brugger S, Anbarchian T, Kim JK, Lee YJ, Lyons KM. The type I BMP receptor ACVR1/ALK2 is required for chondrogenesis during development. *J Bone Min Res*. 2015 in press.
17. Copp AJ, Greene ND, Murdoch JN. The genetic basis of mammalian neurogenesis. *Nat Rev Genet*. 2003; 4:784–93. [PubMed: 13679871]
18. Bond AM, OGB, Kessler JA. The dynamic role of bone morphogenetic proteins in neural stem cell fate and maturation. *Dev Neurobiol*. 2012; 72:1068–84. [PubMed: 22489086]
19. Le Dreau G, Marti E. The multiple activities of BMPs during spinal cord development. *Cell Mol Life Sci*. 2013; 70:4293–305. [PubMed: 23673983]
20. Huang X, Saint-Jeannet JP. Induction of the neural crest and the opportunities of life on the edge. *Dev Biol*. 2004; 275:1–11. [PubMed: 15464568]
21. Kuroda H, Wessley O, De Robertis EM. Neural induction in *Xenopus*: requirement for ectodermal and endomesodermal signals via chordin, noggin, beta-catenin, and cerberus. *PLoS Biology*. 2004; 2:e92. [PubMed: 15138495]
22. Sauka-Spengler T, Bronner-Fraser M. Evolution of the neural crest viewed from a gene regulatory perspective. *Genesis*. 2008; 46:673–82. [PubMed: 19003930]
23. Anderson RM, Lawrence AR, Scottmann RW, Bachiller D, Klingensmith J. Chordin and noggin promote organizing centers of forebrain development in the mouse. *Development*. 2002; 129:4975–87. [PubMed: 12397106]
24. Liem KF, Tremml G, Roelink H, Jessell TM. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell*. 1995; 82:969–79. [PubMed: 7553857]
25. Lopez-Coviella I, Berse B, Krauss R, Ties RS, Blusztaji JK. Induction and maintenance of the neuronal cholinergic phenotype in the central nervous system by BMP-9. *Science*. 2000; 289:313–6. [PubMed: 10894782]
26. Gu Z, Reynolds EM, Song JJ, et al. The type I serine/threonine kinase receptor ActRIA (ALK2) is required for gastrulation of the mouse embryo. *Development*. 1999; 126:2551–61. [PubMed: 10226013]
27. Rowitch DH, Kriegstein AR. Developmental genetics of vertebrate glial-cell specification. *Nature*. 2010; 468:214–22. [PubMed: 21068830]
28. Mehler MF, Mabie PC, Zhu G, Gokhan S, Kessler JA. Developmental changes in progenitor cell responsiveness to bone morphogenetic proteins differentially modulate progressive CNS lineage fate. *Dev Neurosci*. 2000; 22:74–85. [PubMed: 10657700]
29. Nakashima K, Yanagisawa M, Arakawa H, et al. Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. *Science*. 1999; 284:479–82. [PubMed: 10205054]

30. Rajan P, Panchision DM, Newell LF, McKay RD. BMPs signal alternatively through a SMAD or FRAP-STAT pathway to regulate fate choice in CNS stem cells. *J Cell Biol.* 2003; 161:911–21. [PubMed: 12796477]
31. Bertrand N, Castro DS, Guillemot F. Proneural genes and the specification of neural cell types. *Nat Rev Neurosci.* 2002; 3:517–30. [PubMed: 12094208]
32. Nakashima K, Takizawa T, Ochiai W, et al. BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis. *Proc Natl Acad Sci USA.* 2001; 98:5868–73. [PubMed: 11331769]
33. Shore EM, Kaplan FS. Inherited human diseases of heterotopic bone formation. *Nat Rev Rheumatol.* 2010; 6:518–27. [PubMed: 20703219]
34. Kaplan FS, Le Merre M, Glaser DL, et al. Fibrodysplasia ossificans progressiva. *Best Pract Res Clin Rheumatol.* 2008; 22:191–205. [PubMed: 18328989]
35. Shore E, Kaplan FS. Role of altered signal transduction in heterotopic ossification and fibrodysplasia ossificans progressiva. *Curr Osteoporos Rep.* 2011; 9:83–8. [PubMed: 21340697]
36. Kaplan FS, Tabas JA, Gannon FH, Finkel G, Hahn GV, Zasloff MA. The histopathology of fibrodysplasia ossificans progressiva. An endochondral process. *J Bone Joint Surg Am.* 1993; 75:220–30. [PubMed: 7678595]
37. Lefebvre V, Bhattaram P. Vertebrate skeletogenesis. *Curr Topics Dev Biol.* 2010; 90:291–317.
38. Barnett CP, Dugar M, Haan EA. Late-onset variant fibrodysplasia ossificans progressiva leading to misdiagnosis of ankylosing spondylitis. *Am J Med Genet.* 2011; 155A:1492–5. [PubMed: 21567927]
39. Culpert, AL.; Chakkalakal, SA.; Convente, MR.; Lounev, VY.; Kaplan, FS.; Shore, EM. Fibrodysplasia (myositis) ossificans progressiva. In: Thakker, RV.; Whyte, MP.; Eisman, JA.; Igarashi, T., editors. *Genetics of Bone Biology and Skeletal Disease.* New York: Academic Press; 2013. p. 375-93.
40. Kaplan FS, Kobori JA, Orellana C, et al. Multi-system involvement in a severe variant of fibrodysplasia ossificans progressiva (ACVR1 c.772G>A; R258G): A report of two patients. *Am J Med Genet.* 2015; 167:2265–71. [PubMed: 26097044]
41. Kaplan FS, Xu M, Seemann P, et al. Classic and atypical Fibrodysplasia Ossificans Progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. *Hum Mutat.* 2009; 30:379–90. [PubMed: 19085907]
42. Deirmengian GK, Hebela NM, O'Connell M, Glaser DL, Shore E, Kaplan FS. Proximal tibial osteochondromas in patients with fibrodysplasia ossificans progressiva. *J Bone Joint Surg.* 2008; 90:366–74. [PubMed: 18245597]
43. Whyte MP, Wenkert D, Demertzis JL, DiCarlo EF, Westenberg E, Mumm S. Fibrodysplasia Ossificans Progressiva: middle-age onset of heterotopic ossification from a unique missense mutation (c.974G > C, p.G325A) in ACVR1. *J Bone Min Res.* 2012; 27:729–37.
44. Hebela N, Shore E, Kaplan FS. Three pairs of monozygotic twins with fibrodysplasia ossificans progressiva. *Clin Rev Bone Min Met.* 2005; 3:205–8.
45. Bagarova J, Vonner AJ, Armstrong KA, et al. Constitutively active ALK2 receptor mutants require type II receptor cooperation. *Mol Cell Biol.* 2013; 33:2413–24. [PubMed: 23572558]
46. Fukuda T, Kohda M, Koanmata K, et al. Constitutively activated ALK2 and increased SMAD1/5 cooperatively induce bone morphogenetic protein signaling in Fibrodysplasia Ossificans Progressiva. *J Biol Chem.* 2009; 284:7149–56. [PubMed: 18684712]
47. Groppe JC, Shore E, Kaplan FS. Functional modeling of the ACVR1 (R206H) mutation in FOP. *Clin Orth Relat Res.* 2007; 462:87–92.
48. Shore E, Xu M, Feldman GJ, et al. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nature Genet.* 2006; 38:525–7. [PubMed: 16642017]
49. Ohte S, Shin M, Sasanuma H, et al. A novel mutation in ALK2, L196P, found in the most benign case of fibrodysplasia ossificans progressiva activates BMP-specific intracellular signaling equivalent to a typical mutation, R206H. *Biochem Biophys Res Commun.* 2011; 407:213–8. [PubMed: 21377447]

50. Gregson CL, Hollingworth P, Williams M, et al. A novel ACVR1 mutation in the glycine/serine-rich domain found in the most benign case of a fibrodysplasia ossificans progressiva variant reported to date. *Bone*. 2011; 48:654–8. [PubMed: 21044902]
51. Shen Q, Little SC, Xu M, et al. The fibrodysplasia ossificans progressiva R206H ACVRa mutation activates BMP-independent chondrogenesis and zebrafish embryo ventralization. *J Clin Invest*. 2009; 119:3462–72. [PubMed: 19855136]
52. Fukuda T, Scott G, Komatsu Y, et al. Generation of a mouse with conditionally activated signaling through the BMP receptor, ALK2. *Genesis*. 2006; 44:159–67. [PubMed: 16604518]
53. Brunet LJ, McMahon JA, McMahon AP, Harland RM. Noggin, cartilage morphogenesis, and joint formation in the mammalian skeleton. *Science*. 1998; 280:1455–7. [PubMed: 9603738]
54. St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev*. 1999; 13:2076–86.
55. van Dinther M, Visser N, de Gorter DJJ, et al. 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 Min Res*. 2010; 25:1208–15.
56. Culbert AL, Chakkalakal SA, Theosmy EG, Breennan TA, Kaplan FS, Shore EM. Alk2 regulates early chondrogenic fate in Fibrodysplasia Ossificans Progressiva heterotopic endochondral ossification. *Stem Cells*. 2014; 32:1289–300. [PubMed: 24449086]
57. Chakkalakal SA, Zhang D, Culbert AL, et al. An Acvr1 R206H knock-in mouse has Fibrodysplasia Ossificans Progressiva. *J Bone Min Res*. 2012; 27:1746–56.
58. Shimono K, Tung W-E, Macolino C, et al. Potent inhibition of heterotopic ossification by nuclear retinoic acid receptor- γ agonists. *Nature Med*. 2011; 17:454–60. [PubMed: 21460849]
59. Yu PB, Deng DY, et al. BMP type I receptor inhibition reduces heterotopic ossification. *Nat Med*. 2008; 14:1363–9. [PubMed: 19029982]
60. Kaplan FS, Shore EM, Pignolo RJ. The medical management of Fibrodysplasia Ossificans Progressiva: current treatment considerations. *Clin Proc Intl Clin Consort FOP*. 2011; 4:1–100.
61. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacol Rev*. 2004; 56:387–437. [PubMed: 15317910]
62. DiCesare PE, Nimni ME, Pen L, Yazdi M, Cheung DT. Effects of indomethacin on demineralized bone-induced heterotopic ossification in the rat. *J Orthop Res*. 1991; 9:855–61. [PubMed: 1919848]
63. Sims NA, Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *BoneKey Reports*. 2014; 3:481. [PubMed: 24466412]
64. Tang Y, Wu X, Lei W, et al. TGB-beta1-induced migration of bone mesenchymal stem cells couples bone resorption to formation. *Nat Med*. 2009; 15:757–65. [PubMed: 19584867]
65. Brantus JF, Meunier PJ. Effects of intravenous etidronate and oral corticosteroids in fibrodysplasia ossificans progressiva. *Clin Orthop*. 1998; 346:117–20. [PubMed: 9577418]
66. Schuetz P, Mueller B, Christ-Crain M, Dick W, Haas H. Amino-bisphosphonates in heterotopic ossification: first experience in five consecutive cases. *Spinal Cord*. 2005; 43:604–10. [PubMed: 15867938]
67. Wood J, Bonjean K, Ruetz S, et al. Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. *J Pharmacol Exp Therapy*. 2002; 302:1055–61.
68. Kronenberg HM. Developmental regulation of the growth plate. *Nature*. 2003; 423:332–6. [PubMed: 12748651]
69. Kan L, Liu Y, McGuire TL, et al. Dysregulation of local stem/progenitor cells as a common cellular mechanism for heterotopic ossification. *Stem Cells*. 2009; 27:150–6. [PubMed: 18832590]
70. Gannon FH, Glaser DL, Caron R, Thompson LDR, Shore E, Kaplan FS. Mast cell involvement in fibrodysplasia ossificans progressiva. *Hum Pathol*. 2001; 32:842–8. [PubMed: 11521229]
71. Pignolo RJ, Suda RK, Kaplan FS. The fibrodysplasia ossificans progressiva lesion. *Clin Rev Bone Min Met*. 2005; 5:195–200.

72. Nogginuera A, Ros JB, Pavia C, et al. Bisphosphonates, a new treatment for glucocorticoid-induced osteoporosis in children. *J Pediatr Endocrinol Metab.* 2003; 16:529–36. [PubMed: 12793604]
73. Cuny GD, Yu PB, Laha JK, et al. Structure-activity relationship study of bone morphogenetic protein (BMP) signaling inhibitors. *Bioorg Med Chem Lett.* 2008; 18:4388–92. [PubMed: 18621530]
74. Yu PB, Hong CC, Sachidanandan C, et al. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat Chem Biol.* 2008; 4:33–41. [PubMed: 18026094]
75. Mohedas AH, Xing X, Armstrong KA, Bullock AN, Cuny GD, Yu PB. Development of an ALK2-biased BMP type I receptor kinase inhibitor. *ACS Chem Biol.* 2013; 8:1291–302. [PubMed: 23547776]
76. Sanvitale CE, Kerr G, Chaikuad A, et al. A new class of small molecule inhibitor of BMP signaling. *PLoS ONE.* 2013; 8:e62721. [PubMed: 23646137]
77. Evans RM, Mangelsdorf DJ. Nuclear receptors, RXR, and the big bang. *Cell.* 2014; 157:255–66. [PubMed: 24679540]
78. Weston AD, Blumberg B, Underhill TM. Active repression by unligated retinoid receptors in development: less is sometimes more. *J Cell Biol.* 2003; 161:223–8. [PubMed: 12719467]
79. Weston AD, Chandraratna RAS, Torchia J, Underhill TM. Requirement for RAR-mediated gene repression in skeletal progenitor differentiation. *J Cell Biol.* 2002; 158:39–51. [PubMed: 12105181]
80. Pacifici M, Cossu G, Molinaro M, Tato' F. Vitamin A inhibits chondrogenesis but not myogenesis. *Exp Cell Res.* 1980; 129:469–74. [PubMed: 7428831]
81. Shimono K, Morrison TN, Tung W-E, et al. Inhibition of ectopic bone formation by a selective retinoic acid receptor α -agonist: a new therapy for heterotopic ossification? *J Orthop Res.* 2010; 28:271–7. [PubMed: 19725108]
82. Hoffman LM, Garcha K, Karamboulas K, et al. BMP action in skeletogenesis involves attenuation of retinoid signaling. *J Cell Biol.* 2006; 174:101–13. [PubMed: 16818722]
83. Weston AD, Rosen V, Chandraratna RAS, Underhill TM. Regulation of skeletal progenitor differentiation by the BMP and retinoid signaling pathways. *J Cell Biol.* 2000; 148:679–90. [PubMed: 10684250]
84. Yasuhara R, Yuasa T, Williams JA, et al. Wnt/ β -catenin and retinoic acid receptor signaling pathways interact to regulate chondrocyte function and matrix turnover. *J Biol Chem.* 2010; 285:317–27. [PubMed: 19858186]
85. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/ β -catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell.* 2005; 8:739–50. [PubMed: 15866164]
86. Yasuhara R, Ohta Y, Yuasa T, et al. Roles of β -catenin signaling in phenotypic expression and proliferation of articular cartilage superficial zone cells. *Lab Invest.* 2011; 91:1739–52. [PubMed: 21968810]
87. Hind M, Stinchcombe S. Palovarotene, a novel retinoic acid receptor gamma agonist for the treatment of emphysema. *Curr Opin Invest Drugs.* 2009; 10:1243–50.
88. Hatsell SJ, Idone V, Alessi Wolken DM, et al. ACVR1R206H receptor mutation causes fibrodysplasia ossificans progressiva by imparting responsiveness to activin A. *Science Trans Med.* 2015; 7:303ra137.
89. Alemna-Muench GR, Soldevila G. When versatility matters: Activins/inhibins as key regulators of immunity. *Immunol Cell Biol.* 2012; 90:137–48. [PubMed: 21537340]
90. Stiller CA, Nectoux J. International incidence of childhood brain and spinal tumours. *Int J Epidemiol.* 1994; 23:458–64. [PubMed: 7960369]
91. Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: critical review of clinical trials. *Lancet Oncol.* 2006; 7:241–8. [PubMed: 16510333]
92. Sievert AJ, Fisher MJ. Pediatric low-grade gliomas. *J Child Neurology.* 2009; 24:1397–408.
93. Jansen MHA, van Vuurden DG, Vandertop WP, Kaspers GJL. Diffuse intrinsic pontine gliomas: a systematic update on clinical trials and biology. *Cancer Treat Reviews.* 2012; 38:27–35.

94. Louis DN, Perry A, Burger P, et al. International society of neuropathology-Haarlem consensus guidelines for nervous system tumor classification and grading. *Brain Path.* 2014; 24:429–35. [PubMed: 24990071]
95. Epstein F, McCleary EL. Intrinsic brain-stem tumors of childhood: surgical indications. *J Neurosurg.* 1986; 64:11–5. [PubMed: 3941334]
96. Broniscer A, Baker JN, Baker SJ, et al. Prospective collection of tissue samples at autopsy in children with diffuse intrinsic pontine glioma. *Cancer.* 2010; 116:4632–7. [PubMed: 20589749]
97. Kambhampati M, Perez JP, Yadavilli S, et al. A standardized autopsy procurement allows for the comprehensive study of DIPG biology. *Oncotarget.* 2015; 6:12740–7. [PubMed: 25749048]
98. Monje M, Mitra SS, Freret ME, et al. Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. *Proc Natl Acad Sci USA.* 2011; 108:4453–8. [PubMed: 21368213]
99. Paugh BS, Broniscer A, Qu C, et al. Genome-wide analyses identify recurrent amplifications of receptor tyrosine kinases and cell-cycle regulatory genes in diffuse intrinsic pontine glioma. *J Clin Oncol.* 2011; 29:3999–4006.
100. Jones C, Perryman L, Hargrave D. Paediatric and adult malignant glioma: close relative or distant cousins? *Nat Rev Clin Oncol.* 2012; 9:400–13. [PubMed: 22641364]
101. Warren KE, Lillian K, Suuriniemi M, Wang Y, Quezado M, Meltzer PS. Genomic aberrations in pediatric diffuse pontine gliomas. *Neuro Oncol.* 2011; 14:326–32. [PubMed: 22064882]
102. Diaz AK, Baker SJ. The genetic signatures of pediatric high-grade glioma: no longer a one-act play. *Semin Radiat Oncol.* 2014; 24:240–7. [PubMed: 25219808]
103. Wu G, Broniscer A, McEachron TA, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brain stem glioblastomas. *Nat Genet.* 2012; 44:251–3. [PubMed: 22286216]
104. Khuong-Quang DA, Buczkowicz P, Rakopoulos P, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol.* 2012; 124:439–47. [PubMed: 22661320]
105. Bender S, Tang M, Lindroth AM, et al. Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell.* 2013; 24:660–72. [PubMed: 24183680]
106. Wu G, Diaz AK, Paugh BS, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet.* 2014; 46:444–50. [PubMed: 24705251]
107. Grill J, Puget S, Andreuolo F, Philippe C, MacConaill L, Kieran MW. Critical oncogenic mutations in newly diagnosed pediatric diffuse intrinsic pontine glioma. *Pediatr Blood Cancer.* 2012; 58:489–91. [PubMed: 22190243]
108. Buczkowicz P, Hoeman C, Rakopoulos P, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nat Genet.* 2014; 46:451–6. [PubMed: 24705254]
109. Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, et al. Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nat Genet.* 2014; 46:462–6. [PubMed: 24705250]
110. Taylor KR, Mackay A, Truffaux N, et al. Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nat Genet.* 2014; 46:457–61. [PubMed: 24705252]
111. Shi Y, Massague' J. Mechanisms of TGF- β signaling from cell membrane to the nucleus. *Cell.* 2003; 113:685–700. [PubMed: 12809600]
112. Goumans MJ, Valdimarsdottir G, Itoh S, et al. Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGF β /ALK5 signaling. *Mol Cell.* 2003; 12:817–28. [PubMed: 14580334]
113. Massague J. TGF β signaling in context. *Nat Rev Mol Cell Biol.* 2012; 13:616–30. [PubMed: 22992590]
114. Koyama E, Shibukawa Y, Nagayama M, et al. A distinct cohort of progenitor cells participates in synovial joint and articular cartilage formation during mouse limb skeletogenesis. *Dev Biol.* 2008; 316:62–73. [PubMed: 18295755]

115. Lounev VY, Ramachandran R, Wosczyzna M, et al. Identification of progenitor cells that contribute to heterotopic skeletogenesis. *J Bone Joint Surg.* 2009; 91:652–63. [PubMed: 19255227]
116. Ono N, Ono W, Nagasawa T, Kronenberg HM. A subset of chondrogenic cells provides early mesenchymal progenitors in growing bones. *Nat Cell Biol.* 2014; 16:1157–67. [PubMed: 25419849]
117. Wosczyzna MN, Biswas AA, Cogswell cA, Goldhamer DJ. Multipotent progenitors resident in the skeletal muscle interstitium exhibit robust BMP-dependent osteogenic activity and mediate heterotopic ossification. *J Bone Min Res.* 2012; 27:1004–17.
118. Monje M, Mitra SS, Freret ME, et al. hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. *Proc Natl Acad Sci USA.* 2011; 108:4453–8. [PubMed: 21368213]
119. Ma YH, Mentlein R, Knerlich F, Kruse ML, Medhdorn HM, Held-Feindt J. Expression of stem cell markers in human astrocytomas of different WHO grades. *J Neurooncol.* 2008; 86:31–45. [PubMed: 17611714]
120. Ben-Porath I, Thomson MW, Carey VJ, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet.* 2008; 40:499–507. [PubMed: 18443585]
121. Lu QR, Yuk D, Alberta JA, et al. Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. *Neuron.* 2000; 25:317–29. [PubMed: 10719888]
122. Guo X, Wang X-F. Signaling cross-talk between TFG- β /BMP and other pathways. *Cell Research.* 2009; 19:71–88. [PubMed: 19002158]
123. Maurya AK, Tan HL, Souren M, Wang X, Wittbrodt J, Ingham PW. Integration of hedgehog and BMP signaling by the engrailed2a gene in the zebrafish myotome. *Development.* 2011; 138:755–65. [PubMed: 21266411]
124. Saratsis AM, Kambhampati M, Snyder K, et al. Comparative multidimensional molecular analyses of pediatric diffuse intrinsic pontine glioma reveals distinct molecular subtypes. *Acta Neuropathol.* 2014; 127:881–95. [PubMed: 24297113]
125. Brardwaj G, Murdoch B, Wu DY, et al. Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. *Nat Immunol.* 2001; 2:172–80. [PubMed: 11175816]
126. Timmer JR, Wang C, Niswander L. BMP signaling patterns the dorsal and intermediate neural tube via regulation of homeobox and helix-loop-helix transcription factors. *Development.* 2002; 129:2459–72. [PubMed: 11973277]
127. Wacker SA, McNally CL, Durston AJ. The initiation of Hox gene expression in *Xenopus laevis* is controlled by Brachyury and BMP-4. *Dev Biol.* 2004; 266:123–37. [PubMed: 14729483]
128. Costa BM, Smith JS, Chen Y, et al. Reversing HOXA9 oncogene activation by PI3K inhibition: epigenetic mechanisms and prognostic significance in human glioblastoma. *Cancer Res.* 2010; 70:453–62. [PubMed: 20068170]
129. Gaspar N, Marshall L, Perryman L, et al. MGMT-independent temozolomide resistance in pediatric glioblastoma cells associated with a PI3-kinase-mediated HOX/stem cell gene signature. *Cancer Res.* 2010; 70:9243–52. [PubMed: 20935218]
130. Murat A, Migliavacca E, Goria T, et al. Stem cell-related “self-renewal” signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. *J Clin Oncol.* 2008; 26:3015–24. [PubMed: 18565887]
131. Stupp R, Hegi ME, van den Bent MJ, et al. Changing paradigms - An update on the multidisciplinary management of malignant glioma. *Oncologist.* 2006; 11:165–80. [PubMed: 16476837]
132. Bielen A, Perryman L, Box GM, et al. Enhanced efficacy of IGF1R inhibition in pediatric glioblastoma by combinatorial targeting of PDGFR α/β . *Mol Cancer Therapeutics.* 2011; 10:1407–18.
133. Grasso CS, Tang Y, Truffaux N, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nat Med.* 2015; 21:555–9. [PubMed: 25939062]

134. Kan L, Kitterman JA, Procissi D, et al. CNS demyelination in fibrodysplasia ossificans progressiva. *J Neurol.* 2012; 259:2644–55. [PubMed: 22736080]
135. Kitterman JA, Strober JB, Kan L, et al. Eurological symptoms in individuals with fibrodysplasia ossificans progressiva. *J Neurol.* 2012; 259:2636–43. [PubMed: 22752062]
136. Bertamino M, Severino M, Schiaffino MC, et al. New insights into central nervous system involvement in FOP: case report and review of the literature. *Am J Med Genet.* 2015; 167A: 2817–21. [PubMed: 26239063]
137. Koyama E, Golden EB, Kirsch T, et al. Retinoid signaling is required for chondrocyte maturation and endochondral bone formation during limb skeletogenesis. *Dev Biol.* 1999; 208:375–91. [PubMed: 10191052]

Biographies

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Maurizio Pacifci is Director of the Translational Research Program in Pediatric Orthopaedics at the Children's Hospital of Philadelphia (CHOP). He received a doctorate degree in Developmental Biology from the University of Rome, joined the faculty at the University of Pennsylvania where he rose to the rank of Professor, and subsequently moved to CHOP. His long-standing research interests focus on the cellular and molecular mechanisms regulating the formation, growth and morphogenesis of the mammalian skeleton and in particular, the synovial joints. His more recent biomedical research focus is on the pathogenesis of rare and orphan pediatric musculoskeletal disorders and on identification, testing and implementation of therapeutic strategies and tools.

Highlights

- ALK2, the product of *ACVRI*, is a member of the type I bone morphogenetic protein (BMP) receptors
- ALK2 and the BMP signaling pathway have essential roles in skeletal and neural development and homeostasis
- Common activating *ACVRI* mutations have been identified in the pediatric disorders FOP and DIPG
- Analysis and comparison of respective pathogenic mechanisms will help to identify therapeutics for these severe and often fatal conditions

Fig. 1.

Anatomical and histological characteristics of FOP and DIPG. (A–B) Images of an FOP patient showing the progression and increased severity of HO lesions along the back at indicated times. Images are from friendswithfop.com. (C) Histological organization of an HO lesion showing the presence of cartilage (arrow) and bone (double arrow) and reflecting the endochondral nature of HO in FOP. Section was stained with hematoxylin & eosin. (D) CT scans from a DIPG patient showing presence of the tumor mass in the pons (arrows). Images are from northwestern.edu/saratsis/.

Fig. 2.
Schematic showing the number and location of missense mutations in *ACVR1* reported so far in DIPG and FOP. Note that all mutations found in DIPG tumors have been found in FOP patients with the exception of G328V (in red).

Fig. 3.

Schematics depicting possible roles of mutant ACVR1/ALK2 in the pathogenesis of FOP and DIPG. In FOP, mutations in *ACVR1* are known to be necessary and sufficient to cause HO and skeletal problems typical of FOP. The mutations are likely to occur during gametogenesis or very early embryogenesis, and eventually work in concert with local ligands and inflammation (indicated by *) to initiate and sustain a cascade of events that start with recruitment of progenitor cells and culminate with formation of endochondral bone at extraskelatal sites over postnatal life. The various gene regulators controlling each step of the HO process are those previously shown to normally regulate endochondral bone formation in embryos and growing organisms and are assumed to be operating in HO as well. In DIPG, mutations in *ACVR1* are currently thought to be not sufficient to cause tumor formation, and possible ligands for the mutant receptors remain unknown as well (indicated by **). The four pathogenic pathways shown here to which mutant *ACVR1* could contribute are based on: previous extensive studies on genomic landscapes and transcriptome analyses in cohorts of DIPG patients; and roles that wild type ACVR1 is known to have in other developmental processes and systems. These pathogenic pathways are meant to suggest possible gene interactions that mutant *ACVR1* could have with other known DIPG gene mutations/alterations and what specific cell functions and behaviors could in turn be affected.