Am J Cancer Res 2016;6(11):2431-2447
www.ajcr.us /ISSN:2156-6976/ajcr0041138

Review Article

Activin receptor-like kinases: a diverse family playing an important role in cancer

Holli A Loomans[1], Claudia D Andl[2]

[1]*Department of Cancer Biology, Vanderbilt University, Nashville, TN, USA;* [2]*Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, USA*

Received October 3, 2016; Accepted October 12, 2016; Epub November 1, 2016; Published November 15, 2016

Abstract: The role and function of the members of the TGFβ superfamily has been a substantial area of research focus for the last several decades. During that time, it has become apparent that aberrations in TGFβ family signaling, whether through the BMP, Activin, or TGFβ arms of the pathway, can result in tumorigenesis or contribute to its progression. Downstream signaling regulates cellular growth under normal physiological conditions yet induces diverse processes during carcinogenesis, ranging from epithelial- to-mesenchymal transition to cell migration and invasion to angiogenesis. Due to these observations, the question has been raised how to utilize and target components of these signaling pathways in cancer therapy. Given that these cascades include both ligands and receptors, there are multiple levels at which to interfere. Activin receptor-like kinases (ALKs) are a group of seven type I receptors responsible for TGFβ family signal transduction and are utilized by many ligands within the superfamily. The challenge lies in specifically targeting the often-overlapping functional effects of BMP, Activin, or TGFβ signaling during cancer progression. This review focuses on the characteristic function of the individual receptors within each subfamily and their recognized roles in cancer. We next explore the clinical utility of therapeutically targeting ALKs as some have shown partial responses in Phase I clinical trials but disappointing outcomes when used in Phase II studies. Finally, we discuss the challenges and future directions of this body of work.

Keywords: TGFβ, Activin, BMP, targeted therapy

Introduction

The TGFβ superfamily has long been of interest in elucidating the mechanisms of normal physiological development and the development of cancer. This family portrays a seemingly simplistic mechanism of action: the ligand binds a type II receptor dimer, which then forms a tetramer with a type I receptor dimer and transduces a signal through Smad2/3 or Smad1/5/8, ultimately driving nuclear transcription. However, there are numerous family members and several layers of regulation, which complicate the effects following signaling initiation.

The TGFβ superfamily is made up of greater than 30 ligands, which are subdivided into further groups according to structural and mechanistic similarities: transforming growth factor beta ligands (TGFβ), bone morphogenic proteins (BMPs), activins, growth and differentiation factors (GDFs), and anti-Müllerian hormones (AMH) (reviewed in [1, 2]). In contrast to the large family of signaling ligands, there are a small number of receptors to transduce these signals: five type II receptors and seven type I receptors (reviewed in [3]). As there are substantially more ligands than available receptors, there is overlap in the combination of ligands and receptors. Though the focus of this review is on the type I receptors that transduce these signals, it is important to recognize the magnitude of inputs coming into these receptors, resulting in a diverse set of biological outputs, including cell growth suppression, epithelial to mesenchymal transition, and migration and invasion.

Type I receptors

The type I receptors, known collectively as Activin receptor-like kinases (ALKs), are more similar to each other than to the type II receptors, hence their separate classification. The

two classes share only approximately 40% amino acid sequence similarity [4]. In humans, seven ALKs have been identified. This class of kinases is approximately 40-60% homologous and share structural elements including a cysteine- rich extracellular domain with glycosylation site, a transmembrane domain, and a cytoplasmic tail with an active serine/threonine kinase domain [5-8]. Due to their similarities, or, potentially, their differences, the type I receptors form hetero-tetrameric complexes [9, 10]. This results in differential downstream signaling and translational control through pairing with various type II receptors [10]. As there are substantial differences in the biological and functional effects of the type I receptors, the focus of this review will describe their primary function as well as their involvement in cancer and therapeutic potential. However, though this review will focus on ALKs, it is important to note the TGFβ type II receptors, as they play a necessary part in this signaling cascade. The tumor suppressive function of the TGFβ type II receptors have been extensively documented. Several studies have illustrated the presence of TGFβ type II receptor mutations and inactivations. Of particular note, approximately 90% of microsatellite (MS) unstable and 15% of MS stable colorectal cancers have *TGFBR2* inactivating mutations [11]. *TGFBR2* inactivation is more frequently observed in MS unstable cancer types, such as gastric cancer and glioma, than in MS stable cancer [12]. Interestingly, this does not appear to be a phenomenon that occurs among ALKs.

In contrast to the previously stated MS unstable colorectal cancer observations, individuals suffering from hereditary nonpolyposis colorectal cancer, which frequently have *TGFBR2* inactivating mutations, often have overall better outcomes than those with sporadic colorectal cancer, suggesting a protective effect of *TGFBR2* inactivation in these cases [12, 13] (for a more complete review of the literature, please see [12, 14]). The potential duality of function of the TGFβ family receptors is not unique and is a characteristic that translates to ALKs.

As there are differences in the biological and functional effects of the ALKs, the focus of this review will describe their primary tasks as well as the involvement of ALKs in cancer and therapeutic potential.

ALK1

Function

ALK1 (ACVL1) has been well studied for its role in vasculogenesis. ALK1 primarily acts as a BMP receptor, due to the high binding affinity of BMP9 and BMP10, which leads to the type II receptor/ALK1 complex formation. Upon activation, ALK1 signals via Smad1/5/9 most commonly in endothelial cells, contributing to both angiogenesis and lymphatic vessel formation [15-17]. Interestingly, TGFβ has also been found to induce Smad1/5/9 signaling in endothelial cells, however the co-receptor endoglin and ALK5 are required for full activation [18]. During wound healing, ALK1 expression increases to induce blood vessel branching and, upon wound closure, its expression is downregulated [19]. Inhibition of endothelial ALK1 signaling through the use of an ALK1 neutralizing antibody substantially inhibits vasculogenesis and angiogenesis, even when growth factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), are present [20].

Interestingly, some studies have indicated that ALK1/Smad1/5/9 works synergistically with the Notch pathway to regulate angiogenesis [21]. Rostama and colleagues found that Delta-like ligand 4 (Dll4)/Notch with BMP9/ALK1 activation induces cell quiescence through p21 and thrombospondin-1, as well as induces the expression of Hey genes in lung endothelial cells. Additionally, upon loss of Dll4, ALK1/Smad1/5/9 becomes upregulated, therefore compensating for the loss of Notch signaling [21]. Similarly, treatment of primary human endothelial cells with BMP9 induces Hey1 and Hey2 genes through cooperation with Notch, however treatment with soluble ALK1 inhibited expression, demonstrating the close relationship of these two pathways [22].

Loss of function of ALK1 is a primary cause of autosomal dominant vascular dysplasia syndrome, as known as hereditary hemorrhagic telangiectasia type 2 (HHT2) [20]. With an incidence of 1 in 8000, approximately 80-90% of HHT2 cases have mutations in ALK1 or endoglin, a TGFβ family co-receptor [15]. Mutation of ALK1 in HHT2 results in a haploinsufficency, where the affected ALK1 allele can then induce

Table 1. Comprehensive list of the inhibitors discussed in this review

Receptor Target	Inhibitor	Use	Reference
ALK1	ALK1-Fc	Laboratory	[19]
	PF-03446962	Phase I	[16, 27]
	Dalantercept/ACE-041	Phase I/II	[23-25]
ALK2	DMH1	Laboratory	[40]
	K02288	Laboratory	[44]
		Approved –	
	LDN-212854	FOP treatment	[43]
ALK3	LDN-193189	Laboratory	[64]
	DMH2	Laboratory	[64]
	VU0465350	Laboratory	[64]
ALK4	SB-431542	Laboratory	[85]
	SB-505124	Laboratory/Phase I	[84, 107]
ALK5	SB-431542	Laboratory	[85, 105, 108]
	EW-7197	Laboratory	[106]
	SB-505124	Laboratory/Phase I	[107, 109]
	LY-2157299	Phase I	[84, 107]
	GW6604	Phase I	[107]
ALK7	SB-431542	Laboratory	[85]
	SB-505124	Laboratory/Phase I	[84, 107]

mRNA synthesis or degradation of the non-functional protein [15].

Role in cancer and therapeutic potential

As ALK1 has a well-established role in vasculogenesis, as described above, investigating the potential contribution of ALK1 in cancer seems like a logical step. ALK1 global knockout mice are phenotypically similar to BMP9 knockout mice, presenting with enlarged lymphatic vessels and development of cancer [17]. ALK1 expression is induced in the vasculature of breast tumors [19]. Several strategies have been explored to block ALK1 signaling in endothelial cells, thereby downregulating or inhibiting tumor angiogenesis. In vivo, the extracellular domain of ALK1, coupled to a mouse-derived Fc region (ALK1-Fc), has been used as a ligand trap for BMP9/10, thus blocking their binding to endothelial ALK1 [19]. Similarly, dalantercept (ACE-041) is a soluble form of ALK1 that binds to BMP9/10, therefore preventing the activation of endogenous ALK1 and inhibiting signaling complex formation [23, 24].

Dalantercept/ACE-041 showed potential in a Phase I dose-escalation study, where 14 of 29 patients had partial response or stable disease [24]. However, when it was used in Phase II for recurrent or persistent endometrial carcinoma, it was deemed ineffective as a single-agent therapeutic [25]. Combination therapy of dalantercept with the vascular endothelial growth factor receptor (VEGFR) inhibitor sunitinib has shown some promise in metastatic renal cell carcinoma in vivo. When combined with sunitinib, dalantercept induced tumor necrosis, stifled cellular growth and revascularization, and downregulated the expression of pro-angiogenic genes, as well as endothelial cell- specific Notch pathway genes [26].

An additional approach to blocking ALK1 signaling has been through the use of a monoclonal antibody specifically targeting ALK1. PF-03446962 prevents BMP and TGFβ ligands from binding to the ALK1 extracellular domain [16]. It was tested in a Phase I study for advanced solid tumors and has since been approved for use in colorectal cancer, mesothelioma, and endometrial cancer [16, 23]. Most recently, PF- 03446962 was used in a Phase I study for hepatocellular carcinoma. While the majority of patients had adverse effects related to the treatment, 50% of those treated had stable disease, however, no complete or partial responses were observed [27]. Collectively, the studies have indicated that ALK1 may be a feasible therapeutic target for advanced solid tumors. For a complete list of ALK1 inhibitors and additional inhibitors discussed in this review, please refer to **Table 1**.

ALK2

Function

ALK2 (ACVR1) is a bone fide BMP receptor. Various TGFβ, Activin, and BMP ligands, such as BMP9 and Activin B, have been found to induce ALK2 signaling [28, 29]. In Leydig cells, which are found in the testes, ALK2 can inhibit Activin signaling by blocking Activin A access to the type II receptor by forming a type II/type I recep-

tor complex in the absence of ligand [30]. When it acts in this manner, ALK2 has an inhibitory function. Interestingly, induction of signaling through non-TGFβ family ligands has been noted. For example, Tsai and colleagues found that stress-induced phosphoprotein 1 (STIP1) can bind directly to ALK2, independent of a type II receptor, to induce downstream Smad signaling [28].

Primarily studied for its role in osteogenesis and chondrogenesis, ALK2 is required for chondrocyte proliferation and differentiation [31]. This role has been illustrated using an ALK2 knockout mouse model, in which ALK2 was conditionally deleted in cartilage. These mice show defects in bone formation, as observed by shortened cranial bases and hypoplastic cervical vertebrae. Adult ALK2 conditional knockout mice develop progressive kyphosis, or convex curvature of the spine [31].

The importance of ALK2 in osteogenesis is fully grasped in the development of fibrodysplasia ossicans progressive (FOP). FOP is a sporadic, rare disease (incidence of 1 in 2 million) that is characterized by progressive ossification of muscles, tendons, ligaments, and connective tissues [32, 33]. The progression of FOP results in chronic pain and growth impediments, often leading to difficulty breathing and, ultimately, death [33]. Gain-of-function mutations in ALK2 have been identified as the cause of FOP. Approximately 50% of the identified ALK2 mutations occur in the GS activating domain, a serine/threonine rich sequence near the kinase domain of these type I receptors [33]. Of the GS mutations, the R206H mutation comprises approximately 90% [34]. Though many of the ALK2 mutations occur in the same region, genotype-phenotype correlations seem to exist, as the clinical presentation of FOP varies depending on the ALK2 mutation [33]. Interestingly, the type II receptor is required for the gain-of-function effect [35]. Therefore, it stands to reason that not only may the location of the ALK2 mutation dictate the severity of FOP, but also the expression level of the BMP type II receptor may contribute to its phenotype.

Role in cancer and therapeutic potential

Few examples of alterations in ALK2 signaling are have been found in the context of cancer. One of the best-known examples occurs in diffuse intrinsic pontine glioma (DIPG), a rare type of childhood tumor. Buczkowicz and colleagues found that approximately 20% of DIPG tumor samples had similar mutations to those found in FOP, indicating ALK2 gain-of-function [36]. Hyperactivation of ALK2 signaling has also been noted in ovarian cancer. ALK2 activation, via autocrine BMP9 signaling, induces transcription of ID1 and ID3, thus increasing proliferation [28, 37]. Though BMP9 can also signal through ALK1, as discussed above, Herrera and colleagues found that this proliferative phenotype was specific to ALK2 signaling in immortalized ovarian surface epithelial cells and ovarian cancer lines [37].

In contrast, maintaining ALK2 signaling has been indicated as a regulator of tumor suppression. Olsen et al. found that treatment of primary multiple myeloma samples with BMP9 resulted in signaling through ALK2 and the induction of apoptosis [38]. In melanoma, treatment with BMP7 upregulates ALK2, inducing mesenchymal-to- epithelial transition via downregulation of Twist, leading to an overall reduction of invasion [39].

Specifically targeting ALK2 in therapeutics has proven challenging. DMH1 is a dorsomorphin analog and selective inhibitor of ALK2 [40]. It reduces the ability of ALK2 to phosphorylate Smad1/5/9 without affecting other kinases such as ALK5, AMPK, or VEGFR [40-42]. DMH1, however, has not been reported for use in the clinic. An additional dorsomorphin analog, LDN-212854, has been used as a means to treat or prevent FOP, yet this inhibitor also non-specifically inhibits ALK1 and ALK3 [43]. Thus far, the most effective ALK2-inhibition strategy has been through the use of K02288, a 2-aminopyridine compound with high affinity for both ALK1 and ALK2. This inhibitor binds to a conserved binding pocket in ALK1 and ALK2, which results in reduced angiogenesis and vessel sprout in vitro [44]. Targeting ALK2, along with the non-specific inhibition of the other ALK kinases ALK2 and ALK3, has been shown as an effective strategy in vitro, one that may show efficacy upon further clinical testing.

ALK3

Function

Though some functions of ALK3 (BMPR1A) suggest similarity to ALK2, this protein has substantial sequence similarity with a different ALK family member, ALK6 [45]. Currently, it is known

that many BMPs (BMP2, 4, 5, 6, 7, 8, 14, 15), GDF6, GDF7, and AMH can bind to ALK3 with some affinity to initiate differential downstream action (reviewed in [46]). Global knockout of ALK3 is embryonic lethal [47]. ALK3 is expressed in cells of the osteo lineage and bone marrow and is necessary for post-natal bone formation; as such, it is suggested that the main function of ALK3 is osteogenesis [32]. Mice with conditional knockout of ALK3 in cartilage lack growth of the long bones, however this tissue gets replaced with bone-like tissue, supporting a role of ALK3 in cell fate and osteogenesis [48]. Conditional deletion of Alk3 from osteoblasts produces a similar phenotype. These mice have increased bone formation in the trabecular bone, vertebrae, tail, and ribs, coupled with a reduction in osteoclastogenesis [49].

Unlike ALK2, ALK3 has additional critical roles in development. Deletion of ALK3 in Xenopus results in dorsalized embryos and defects in the eye [50]. Myocardial- and neural crest-specific knockouts of ALK3 show drastic developmental defects (reviewed in [51]). Epicardial-specific deletion of ALK3 in mice results in atypical developmental of the atrioventricular sulcus and annulus fibrosis within the cardia [52]. Additionally, when ALK3 is deleted early (at weaning or early adulthood) from the foregut, mice have improper gastric patterning, as well as a reduced number of parietal cells and increased number of endocrine cells [53].

Affecting another area of the gastrointestinal tract, the loss of or mutations in BMPR1A/ALK3 has been associated with the development of juvenile polyposis syndrome (JPS), a hereditary condition that is characterized by the presence of hamartomatous polyps and associated with an increased risk for colorectal cancer. Germline mutations in ALK3 are found in approximately 20% of JPS cases, while 45% of cases have mutations in ALK3 and/or the co-Smad, Smad4, therefore contributing to loss of ALK3 signaling [54, 55]. Additionally, case reports of JPS have reported ALK3 mutations occurring independently of Smad4 mutations [56, 57].

Role in cancer and therapeutic potential

Loss of ALK3 is best known to contribute to increased risk for colorectal cancer (CRC). As described above, individuals with JPS, which is associated with loss of ALK3 signaling, have an increased risk of cancer development, particularly CRC. However, it has also been noted that loss of ALK3 has also been associated increased risk of esophageal carcinoma, adrenal hamartoma, and Wilm's tumors [56, 58]. Without JPS as a predisposition, mutations in ALK3, along with several other mismatch repair genes including Smad4, account for less than 5% of CRC [59]. Chang and colleagues found similar results. In a cohort of 103 patients, one patient was found to have a de novo mutation in ALK3 [60]. Similarly to CRC, individuals with loss of ALK3, but unaffected Smad4 expression in pancreatic ductal adenocarcinoma, have significantly poorer survival compared to those who are ALK3-positive [61].

In contrast to loss of signaling contributing to colorectal cancer, abrogation of ALK3 in other cancer contexts appears to promote aggressiveness. Pickup and colleagues found that conditional knockout of ALK3 in breast cancer cell lines results in delayed tumor onset in vivo, however these cells acquire more mesenchymal markers. Analysis of patient data from The Cancer Genome Atlas indicated that individuals with high ALK3 expression had overall poorer survival, regardless of subtype [62].

Additionally, BMP2-induced ALK3 signaling in liposarcoma has been associated with increased extracellular matrix remodeling, disease progression, and, therefore, poorer patient outcome [63].

As the role of ALK3 in cancer is a double-edged sword, the feasibility of targeting this receptor in vivo remains uncertain. Currently, the only reported ALK3 inhibitors in use are LDN-193189, DMH2, and VU0465350. These inhibitors have been used to treat liver disease by enhancing liver regeneration [64].

ALK4

Function

ALK4 (ACVR1B) is a versatile receptor that has a critical role in development. In Xenopus, ALK4 activates both sides of the developmental pathway: the TGFβ-driven left side with the ligands Xnrl and derriere and the BMP-driven right side with the ligand Vgl [65]. This activation modu-

lates mesoderm induction and dorsoanterior/ventroposterior development during primary axis formation [65].

Constitutive activation of ALK4 induces Xenopus mesodermal and dorsoanterior markers, similarly to Activin expression models [66].

In mouse models, global knockout of Alk4 (Acvr1b) is embryonic lethal due to developmental impairment of the epiblast and extraembryonic ectoderm, leading to improper gastrulation [67]. Activation of ALK4 can occur through a multitude of ligands, such as Activins, GDFs, and Nodal [68]. Conditional knockouts of Alk4 in various adult tissues have been generated to analyze the impact of Alk4 systemically. Activin A signaling, mediated via ALK4, has a substantial role in reproduction. Trophoblast invasion is regulated through a canonical ALK4-mediated pathway, where upregulated SNAIL induces MMP-2 expression; knockdown of ALK4 attenuated this effect [69].

Conditional knockout of uterine Alk4 results in subfertility due to defects in placental development [70]. Signaling through ALK4/ALK7 in the male reproductive tract is required for germ cell development and Sertoli cell proliferation [71]. This may be initiated by Nodal or GDFs, along with Cripto, as these mechanisms can trigger downstream phosphorylation of Smad2 [72, 73].

Additional conditional mouse models have been generated to examine the tissue specific effects of ALK4. Squamous cell deletion of Alk4 leads to substantial hair loss, increased epidermal thickness, and growth stunting in approximately 25% of Alk4-null mice. Interestingly, this appears to have a dose-dependent effect, as those with the highest Cre-driven Alk4 expression had the most severe phenotype. Molecularly, Alk4- deleted tissues had increased expression of the transcription factor Lef1, which regulated hair-specific expression of keratin, and increased proliferation [74]. A subsequent conditional model of Alk4 in adult tissues has also been developed, with Alk4 loss observed in skin, liver, spleen, pancreas, and kidney [75].

Role in cancer and therapeutic potential

The best-characterized alterations of ALK4 expression have been noted in pituitary and pancreatic cancers. Alternatively spliced forms of ALK4 have been identified in somatotroph, corticotroph, and nonfunctioning pituitary adenomas, which are generally not found in normal tissue [76]. These splice variants of ALK4 are truncated, lacking the kinase domain and, therefore, cannot propagate anti-proliferative signals [76, 77]. Restoration of full-length ALK4 reverses these effects [77]. Conversely, rather than alternative splicing, pancreatic cancers frequently show ALK4 deletions. Su and colleagues identified loss of heterozygosity of ALK4 in 34% of cancer xenografts and 45% of pancreatic cancer cell lines, supporting the hypothesis that ALK4 acts as a tumor suppressor in this cancer type [78]. In ALK4-positive pancreatic cancer cell lines, invasion could be inhibited through treatment with SB-431542, a chemical inhibitor of ALK4/5/7 [79].

Though the role of ALK4 has not been comprehensively explored in most cancer contexts, several studies have suggested that ALK4 expression can have either oncogenic or tumor suppressive influences. Both prostate cancer cell lines and testicular carcinomas have been shown to retain variable levels of ALK4 expression [80, 81]. Landis and colleagues found that, in ErbB2/Her2/Neu positive tumors, TGFβ signaling through ALK5 was frequently lost, however ALK4-mediated Activin A signaling remained active along the invasive front of the tumor [82]. Similarly, B16 melanoma cells, upon treatment with Activin A, showed dose-dependent regulation of CDH1 and HMGA2 expression. However, in the metastatic cultured B16 cell lines, ALK4 expression was substantially reduced [83].

Targeting ALK4 in therapeutics appears to be, generally, an unfeasible target, as most noted alterations in ALK4 signaling are deletions or inactivations. In some cancers, such as prostate, testicular, or Her2-positive breast cancers, as discussed above, targeting ALK4 may have potential. However, the existing inhibitors not only target ALK4, but the additional type I receptors ALK5 and/or ALK7. SB-505124 and SB-431542 potently inhibit ALK4/5/7, as shown by decreased phosphorylation of Smad2 and Smad1/5/9 [84, 85]. These two inhibitors are not currently used clinically. Additional inhibitors, such as LY-362947, LY-2157299, and SD-208, among others, have also been developed (reviewed in [86]).

ALK5

Function

Similar to the previously discussed receptors, ALK5 (TGFBR1) plays critical roles in development and reproduction. ALK5 has been best characterized as the primary receptor for the TGFβ ligands (TGFβ1, TGFβ2, TGFβ3), however GDF8 and GDF9 have been additionally reported to signal through this receptor [87, 88]. GDF8 can signal via ALK5 to activate Smad3, ERK1/2, and steroidogenic acute regulatory protein (StAR) in granulosa cells. This effect can be blocked by inhibition of ALK5 [87]. GDF9 signals through BMPRII and ALK5 recruiting Smad2 and Smad3 to activate adrenalcortical and Sertoli cells [88]. Both are required for folliculogenesis [87, 88].

ALK5 is required for proper embryonic development; Alk5 knockout mice are embryonic lethal [89]. Even Alk5 mutant mice with a D266A knock-in mutation in the L45 loop, therefore not allowing for Alk5 to phosphorylate Smad2, only survive until E10.5 due to defects in vasculature formation [90]. Targeted deletion of Alk5 in the neural stem compartment is embryonic lethal at E15 due to failure of upper lip palate closure [91]. Because of its embryonic lethality, investigators have utilized conditional knockouts of Alk5 to examine its role in the development of various tissues. Deletion of Alk5 in the endocardium, using Tie2-Cre in vitro and in vivo, demonstrated that this signaling pathway is necessary for not only epithelial-to-mesenchymal transition of the cardiac cells, but also differentiation and maintenance of tight junctions, as observed through downregulation of N-cadherin and VE-cadherin [92]. Additional vascular defects were noted in mice with Alk5 knockout in skin lymphatic endothelial cells. These vascular networks lacked organization and complexity, and were hyperproliferative [93].

Previously, we discussed that ALK5 is necessary for reproductive function. Mice with conditional knockout of Alk5 in the uterus are sterile. Deletion of Alk5 disrupts development of the oviductal diverticula and myometrium. Additionally, these mice have hyperproliferative uteruses and irregular glands [94]. This phenotype is exemplified during implantation, as trophoblasts lack organization, a reduction in the uterine natural killer cell population, and diminished arterial remodeling [95]. These results suggest that ALK5 is necessary to not only transduce TGFβ family signaling, but that it is also needed to mediate part of the uterine immune response. Though loss of Alk5 expression in the uterus results in defective processes, constitutive expression of the receptor is additionally problematic. Conditional gain-of-function of uterine Alk5 resulted in increased myometrium thickness, causing hypermuscularized uteri. In the endometrium, constitutive activity of Alk5 promoted fibroblast differentiation and a smooth muscle gene signature. Interestingly, deletion of Alk5 in the uterus had substantial epithelial effects, while constitutive activation heavily altered the uterine microenvironment [96].

Role in cancer and therapeutic potential

Though research suggests that alterations of ALK5 promote cancer progression, ALK5 alone appears not to be sufficient for this process and needs to cooperate with an oncogenic driver. Examples of this have been explored in breast, colorectal, head and neck squamous (HNSCC), and pancreatic cancers. Landis and colleagues found that phosphorylation of Smad2 was substantially downregulated in a mammary cancer model of ErbB2/Her2/Neu amplification, a result of Alk5 loss [82]. APC is mutated in approximately 70% of sporadic CRC and is often described as the "first hit" [97]. The commonly used APC^{min} mouse model, combined with loss of heterozygosity of Alk5, develop approximately three times more tumors than APC^{min}/Alk5 wild-type mice, indicating that diminished Alk5 signaling can be a second-hit accelerating tumor formation in CRC. Alk5 wild-type and knockout mice alone did not develop CRC tumors [98]. Alterations in ALK5 in CRC frequently occurs through the deletion of three alanines located in a nine alanine repeat, termed TGFBR1*6A [99]. Homozygous variants of TGFBR1*6A has been associated with increased risk of CRC, though a more substantial rate of this deletion has been observed in CRC metastases, compared to primary tumors [99, 100].

In HNSCC, PI3K pathway mutations occur at a frequency of approximately 30% [101]. Of the PI3K pathway mutations, loss of PTEN expression is common [102].

Additionally, HNSCC patient samples and cell lines have marked reduction in ALK5 protein expression. A combination of these two models, knockout of Pten and Alk5, in HNSCC promoted the expansion of the cancer stem cell niche, reduced cellular senescence, and increased cancer-associated inflammation [103]. This association of ALK5 has also been strengthened in a mouse model of pancreatic cancer. Homozygous loss of Alk5, with mutant Kras, led to the development of pre-cancerous lesions at 100% frequency. While only 50% of Alk5 heterozygotes showed pre-cancerous lesions, those that were developed were larger than those in knockout mice [104].

Though loss of ALK5 often confers a tumor cell advantage, ALK5 activation has been noted in osteosarcoma and sex-cord stromal tumors, indicating a dual role for this signaling receptor. Treatment of the osteosarcoma cell line MG63 with TGFβ, which acts primarily through ALK5, induced cellular proliferation via the Smad2/3/4 axis. This effect could be inhibited through treatment with SB-431542 [105]. Additionally, constitutively active Alk5 in ovarian granulosa cells, described previously, promoted the development of sex-cord stromal tumors within two months. These tumors also had elevated expression of the Hedgehog proteins Gli1 and Gli2 [106].

As described above, ALK5 deletion or mutation in tumors appears to be the common form of pathway alteration. That being said, efforts have been directed at inhibiting the ALK5 cascade and, surprisingly, have had some success. Several of these inhibitors such as LY-2157299, SB-505124, and GW6604 have been tested in Phase I and Phase II clinical trials (reviewed in [107]). These inhibitors target the ATP binding pocket of the kinase, removing its signal transduction ability. A limitation of several ALK5 inhibitors is their lack of selectivity between ALKs; for example, SB-431542 inhibits not just ALK5, but also ALK4 and ALK7 [85].

However, these off-target effects of inhibitors can be utilized. Dasatinib, a commonly used Src inhibitor, has also been used in vitro to inhibit ALK5 in PDAC; it has been shown to inhibit Smad2 phosphorylation and cell invasion, similarly to that observed following treatment with the ALK4/5/7 inhibitor SB-431542 [108].

Efforts have been directed at developing ALK5-specific inhibitors. EW-7197 has been used as an in vitro treatment for melanoma. Use of EW-7197 enhanced the number of tumor-infiltrating lymphocytes, particularly CD8+ cytotoxic T cells [107, 109], thereby stimulating the immune response.

ALK6

Function

ALK6 (BMPRIB) acts primarily as a BMP receptor, with preferential binding to BMP2, BMP4, BMP6, BMP7, BMP15 and GDF5, however ligand binding of Müllerian-inhibiting substance (MIS) has also been observed [110, 111]. In Xenopus, the necessity of ALK6 during development has been shown, as loss of ALK6 causes defects in neural crest formation and pigmentation, resulting in an embryonic lethal phenotype [50].

In vertebrate development, expression of ALK6 is tightly controlled, and primarily found in mesenchymal pre-cartilage, chondrocytes and osteoblasts. During osteoblast differentiation, ALK6 expression is upregulated, indicating a role for this protein in bone formation [32]. This is further indicated in the association of ALK6 mutations in the development of brachydactyly type A1 and type A2. Brachydactyly is an autosomal domain disorder affecting the digits. Type A1 is an inherited disorder characterized by malformation of the middle 2-5 fingers, while type A2 is autosomal and characterized by shortening of the index fingers and, sometimes, the first and second toes [112, 113]. Two heterozygous mutations in ALK6 have been identified in association with the development of type A1, which work to halt kinase function [113]. Similarly, linkage analysis of two families identified mutations falling within the GS and kinase domains. Interestingly, only the GS domain mutation rendered the protein kinase-dead, while the kinase domain mutations appeared to have no effect on kinase activity, indicating that these mutations differentially affect ALK6 function [112]. Additional evidence implicating ALK6 in bone formation is the development of the skeletal disorders Grebe dysplasia and acromesomelic du Pan dysplasia [111, 114]. Various mutations affecting the activity of the ALK6 kinase domain have been associated with these disorders [111, 115].

ALK6 expression is highest in the brain, lung, and ovary in adult tissues (reviewed in [45]). In the ovaries, ALK6 is necessary for folliculogenesis. Its expression fluctuates throughout the stages, however, reduced or disrupted ALK6 expression on the granulosa cell surface has been associated with reduced growth of the follicles [116]. Adult sheep with a point mutation in ALK6 have impaired follicle development and an increased ovulation rate [117].

Role in cancer and therapeutic potential

As is a recurrent theme in the TGFβ family, the role of ALK6 in cancer appears to be both tumor promoting and suppressive. Examples of the oncogenic properties of ALK6 have been explored in chronic myeloid leukemia (CML), epithelial ovarian cancer, luminal breast cancer, and colorectal cancer. Upregulation or overexpression of ALK6 has been identified on CML cells, compared to healthy donors. It is suggested that microenvironmental influence of BMP2 and BMP4 contribute to the noted change in ALK6 expression [118]. A similar observation has been made in epithelial ovarian cancers, where patient samples with ALK6 expression had a worse prognosis compared to patients without ALK6 expression [110]. In the investigation of ALK6-ligand interactions in cancer, BMP2 binding and signaling through ALK6, in conjunction with IL-6, has been shown to promote the development of luminal breast cancer through the upregulation of Smad5 and GATA3, as well as downregulation of FOXC1 [119]. Using MCF10A cells, Chapellier and colleagues demonstrated increased colony formation in soft agar and greater ability to form tumors in vivo [120]. Functionally, in vitro knockdown of ALK6 in SW480 CRC cells, which are Smad4 positive, decreased invasion [121].

Though primarily seemingly oncogenic, ALK6 appears to act as a tumor suppressor in gliomas and glioblastomas. Expression of ALK6 is downregulated in various malignant gliomas, including astrocytomas and glioblastomas, compared to normal astrocytes, as measured by mRNA expression and reduced phosphorylation of Smad1/5/9. Re-expression of ALK6 decreased the ability of these cells to have anchorage-independent growth [122]. Additionally, treatment of glioblastoma samples with BMP7 decreased proliferation and sphere number [123]. These studies indicate that, when the ALK6 pathway is active in gliomas and glioblastomas, is acts as a suppressor. Interestingly, the function of this pathway is likely dependent upon the activating ligand, as stimulation with BMP2 and BMP7 appears to have differential effects. That being said, despite having limited therapeutic options in development or available, treatment with BMP ligands themselves shows some potential in vitro [124-126].

ALK7

Function

Little is known regarding ALK7 (ACVR1C). Until recent years, the type II receptor and ligands interacting with ALK7 were unknown [127]. Several ligands have now been identified, including GDF3, Activin B, and Activin AB [128-130]. ALK7 shows high sequence similarity to ALK4 and ALK5, however, the structure of the extracellular domain diverges from the other type I receptors [8, 127]. Similarly to other ALKs, ALK7 substantially impacts development. In Xenopus, active ALK7 is associated with the induction of the mesoendodermal markers [131]. In post-natal development and adulthood, ALK7 is primarily expressed in the central nervous system, where the signaling pathway is suggested to be involved in neuronal proliferation and differentiation, as well as the pancreas and colon [6, 127]. Alk7 knockout mice have been shown to metabolic issues, including reduced insulin sensitivity, impaired glucose tolerance, and enlargement of pancreatic islands [129].

Role in cancer and therapeutic potential

Loss of ALK7 in cancer has been, thus far, consistently associated with poor outcomes. In gallbladder cancer, expression of ALK7 has been associated with better survival compared to patients with ALK7-negative squamous cell, adenosquamous, and adenocarcinomas of the gallbladder [132]. A similar pattern was found in breast cancer, where ALK7 expression becomes lost with increased cancer grade and stage [133]. In vitro utilization of a triple negative breast cancer cell line showed that re-expression of ALK7, along with Activin B treatment, can restore the functional effects of this pathway and inhibit proliferation. A more molecular examination of ALK7 in ovarian cancer has

indicated that ALK7 signaling, via the Smad2/3 axis, can upregulate and downregulate cyclin G2 and Skp1 and Skp2, respectively, whereby inhibiting cell cycle and acting as a tumor suppressor [134]. ALK7 regulation in cancer may be a result of post- transcriptional regulation. Ye and colleagues found that loss of ALK7 in ovarian cancer patient samples was associated with high expression of mir376c. Additionally, high levels of mir376c were found in patients who exhibited chemoresistance. In vitro overexpression of ALK7 could partially overcome cisplatin-induced cell death [135].

As previously discussed, specific inhibition of ALK7 has proven no small feat. Of the current inhibitors, SB-431542 and SB-505124 are the most highly utilized in the laboratory, with SB-505124 being used in Phase I clinical trials [84, 85]. However, as these are not specific inhibitors (also target ALK4 and ALK5), there is no sure way to know if targeting ALK7 is yet beneficial, or if targeting the combination of receptors is more efficacious.

Conclusion

As presented here, signaling of the TGFβ signaling family is mediated by the formation of heteromeric complexes of type I and type II receptors. Downstream signaling occurs upon ligand binding and affects various cellular processes in normal cells with implications for tumorigenesis through the regulation of apoptosis, migration and invasion, angiogenesis and immune response. The promise of using ALKs as therapeutic targets has been shown in successful Phase I clinical trials, yet the challenge lies in restoring homeostasis in the face of multiple overlapping downstream signaling cascades and the potential of off-target effects resulting in serious side-effects. Additional difficulties are met by the similarity of these receptors and the aim to specifically inhibit one type of receptor. Aside from the receptor inhibition as described in this review, other pre-clinical tests aimed to neutralize the ligands. Neutralizing antibodies against TGFβ, 1D11, demonstrated successful suppression of metastasis in a mouse breast cancer model [136]. Clinical trials testing a number of approaches to inhibit TGFβ signaling are recruiting or ongoing (clinicaltrials.gov). Ligand traps consisting of extracellular domains of human ACVR (mostly type 2) have been developed and are valuable in Activin-induced muscle wasting, cachexia, a complication of cancer.

Taken together, although most cancers show alterations of the TGFβ pathway, use of inhibitors has shown some encouraging early results, yet many hurdles have to be overcome before they could be considered for first-line treatments.

Acknowledgements

This work was supported by grants from the National Institute of Health (DK094900, DK091491 to CDA; T32-CA0095193-26, F31-DE025477-01A1, VICTR VR16470 to HAL).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Claudia D Andl, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, 4110 Libra Drive, Building 20, BMS 223, Orlando, FL 32816, USA. Tel: 407-823-1147; E-mail: claudia.andl@ucf.edu

References

[1] Yadin D, Knaus P, Mueller TD. Structural insights into BMP receptors: Specificity, activation and inhibition. Cytokine Growth Factor Rev 2016; 27: 13-34.

[2] Katagiri T, Watabe T. Bone Morphogenetic Proteins. Cold Spring Harb Perspect Biol 2016; 8.

[3] Attisano L, Wrana JL. Signal transduction by members of the transforming growth factor-β superfamily. Cytokine Growth Factor Rev 1996; 7: 327-39.

[4] Wrana JL, Attisano L, Wieser R, Ventura F, Massagué J. Mechanism of activation of the TGFβ receptor. Nature 1994; 370: 341-7.

[5] Kang Y, Reddi AH. Identification and cloning of a novel type I serine/threonine kinase receptor of the TGFβ/BMP superfamily in rat prostate. Biochem Mol Biol Int 1996; 40: 993-1001.

[6] Bondestam J, Huotari MA, Morén A, Ustinov J, Kaivo-Oja N, Kallio J, Horelli-Kuitunen N, Aaltonen J, Fujii M, Moustakas A, ten Dijke P, Otonkoski T, Ritvos O. cDNA cloning, expression studies and chromosome mapping of human type I serine/threonine kinase receptor ALK7 (ACVR1C). Cytogenet Cell Genet 2001; 95: 157-62.

[7] Ryden M, Imamura T, Jornvall H, Belluador N, Neveu I, Trupp M, Okadome T, ten Dijke P, Ibanez CF. A Novel Type I Receptor Serine-Threonine Kinase Predominantly Expressed in the Adult Central Nervous System. J Biochem 1996; 271: 30603-9.

[8] Tsuchida K, Sawchenko PE, Nishikawa SI, Vale WW. Molecular Cloning of a Novel Type I Receptor Serine/Threonine Kinase for the TGFβ Superfamily from Rat Brain. Mol Cell Neuro 1996; 7: 467-78.

[9] Lin SJ, Lerch TF, Cook RW, Jardetzky TS, Woodruff TK. The structural basis of TGFβ, bone morphogenetic protein, and activin ligand binding. Reproduction 2006; 132: 179-90.

[10] de Kroon LM, Narcisi R, Blaney Davidson EN, Cleary MA, van Beuningen HM, Koevoet WJ, van Osch GJ, van der Kraan PM. Activin Receptor-Like Kinase Receptors ALK5 and ALK1 Are Both Required for TGFβ-Induced Chondrogenic Differentiation of Human Bone Marrow-Derived Mesenchymal Stem Cells. PLoS One 2015; 10: e0146124.

[11] Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor β type II receptor gene in colorectal cancer. Cancer Res 1995; 55: 5548-50.

[12] Derynck R, Akhurst RJ, Balmain A. TGFβ signaling in tumor suppression and cancer progression. Nat Genet 2001; 29: 117-29.

[13] Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B, Brattain M, Willson JK. Inactivation of the Type II TGFβ Receptor in Colon Cancer Cells with Microsatellite Instability. Science 1995; 268: 1336-8.

[14] Miyazono K, Ichijo H, Heldin CH. Transforming Growth Factor-β: Latent Forms, Binding Proteins and Receptors. Growth Factors 2009; 8: 11-22.

[15] Alaa el Din F, Patri S, Thoreau V, Rodriguez-Ballesteros M, Hamade E, Bailly S, Gilbert-Dussardier B, Merhi RA, Kitzis A. Functional and splicing defect analysis of 23 ACVRL1 mutations in a cohort of patients affected by Hereditary Hemorrhagic Telangiectasia. PLoS One 2015; 10: e0132111.

[16] Goff LW, Cohen RB, Berlin JD, de Braud FG, Lyshchik A, Noberasco C, Bertolini F, Carpertieri M, Stampino CG, Abbattista A, Wang E, Borghaei H. A Phase I Study of the Anti-Activin Receptor-Like Kinase 1 (ALK-1) Monoclonal Antibody PF-03446962 in Patients with Advanced Solid Tumors. Clin Cancer Res 2016; 22: 2146-54.

[17] Yoshimatsu Y, Lee YG, Akatsu Y, Taguchi L, Suzuki HI, Cunha SI, Maruyama K, Suzuki Y, Yamazaki T, Katsura A, Oh SP, Zimmers TA, Lee SJ, Pietras K, Koh GY, Miyazono K, Watabe T. Bone morphogenetic protein-9 inhibits lymphatic vessel formation via activin receptor-like kinase 1 during development and cancer progression. Proc Natl Acad Sci U S A 2013; 110: 18940-5.

[18] Daly AC, Randall RA, Hill CS. Transforming Growth Factor β-Induced Smad1/5 Phosphorylation in Epithelial Cells Is Mediated by Novel Receptor Complexes and Is Essential for Anchorage-Independent Growth. Mol Cell Biol 2008; 28: 6889-902.

[19] Mitchell D, Pobre EG, Mulivor AW, Grinberg AV, Castonguay R, Monnell TE, Solban N, Ucran JA, Pearsall RS, Underwood KW, Seehra J, Kumar R. ALK1-Fc Inhibits Multiple Mediators of Angiogenesis and Suppresses Tumor Growth. Mol Cancer Ther 2010; 9: 379-88.

[20] Hu-Lowe DD, Chen E, Zhang L, Watson KD, Mancuso P, Lappin P, Wickman G, Chen JH, Wang J, Jiang X, Amundson K, Simon R, Erbersdobler A, Bergqvist S, Feng Z, Swanson TA, Simmons BH, Lippincott J, Casperson GF, Levin WJ, Stampino CG, Shalinsky DR, Ferrara KW, Fiedler W, Bertolini F. Targeting Activin Receptor-Like Kinase 1 Inhibits Angiogenesis and Tumorigenesis through a Mechanism of Action Complementary to Anti-VEGF Therapies. Cancer Res 2011; 71: 1362-73.

[21] Rostama B, Turner JE, Seavey GT, Norton CR, Gridley T, Vary CP, Liaw L. DLL4/Notch1 and BMP9 Interdependent Signaling Induces Human Endothelial Cell Quiescence via P27KIP1 and Thrombospondin-1. Arterioscler Thromb Vasc Biol 2015; 35: 2626-37.

[22] Wöltje K, Jabs M, Fischer A. Serum Induces Transcription of Hey1 and Hey2 Genes by Alk1 but Not Notch Signaling in Endothelial Cells. PLoS One 2015; 10: e0120547.

[23] Gupta S, Gill D, Pal SK, Agarwal N. Activin Receptor Inhibitors-Dalantercept. Curr Oncol Rep 2015; 17: 14.

[24] Bendell JC, Gordon MS, Hurwitz HI, Jones SF, Mendelson DS, Blobe GC, Agarwal N, Condon CH, Wilson D, Pearsall AE, Yang Y, McClure T, Attie KM, Sherman ML, Sharma S. Safety, Pharmacokinetics, Pharmacodynamics, and Antitumor Activity of Dalantercept, an Activin Receptor-like Kinase-1 Ligand Trap, in Patients with Advanced Cancer. Clin Cancer Res 2014; 20: 480-9.

[25] Makker V, Filiaci VL, Chen LM, Darus CJ, Kendrick JE, Sutton G, Moxley K, Aghajanian C. Gynecologic Oncology. Gynecol Oncol 2015; 138: 24-9.

[26] Wang X, Solban N, Khanna P, Callea M, Song J, Alsop DC, Pearsall RS, Atkins MB, Mier JW, Signoretti S, Alimzhanov M, Kumar R, Bhasin

MK, Bhatt RS. Inhibition of ALK1 signaling with dalantercept combined with VEGFR TKI leads to tumor stasis in renal cell carcinoma. Oncotarget 2016; [Epub ahead of print].

[27] Simonelli M, Zucali P, Santoro A, Thomas MB, de Braud FG, Borghaei H, Berlin J, Denlinger CS, Noberasco C, Rimassa L, Kim TY, English PA, Abbattista A, Stampino CG, Carpentieri M, Williams JA. Phase I study of PF-03446962, a fully human monoclonal antibody against activin receptor-like kinase-1, in patients with hepatocellular carcinoma. Annals Oncol 2016; 27: 1782-7.

[28] Tsai CL, Tsai CN, Lin CY, Chen HW, Lee YS, Chao A, Wang TH, Wang HS, Lai CH. Secreted Stress-Induced Phosphoprotein 1 Activates the ALK2-SMAD Signaling Pathways and Promotes Cell Proliferation of Ovarian Cancer Cells. Cell Rep 2012; 2: 283-93.

[29] Canali S, Core AB, Zumbrennen-Bullough KB, Merkulova M, Wang CY, Schneyer AL, Pietrangelo A, Babitt JL. Activin B Induces Noncanonical SMAD1/5/8 Signaling via BMP Type I Receptors in Hepatocytes: Evidence for a Role in Hepcidin Induction by Inflammation in Male Mice. Endocrinology 2016; 157: 1146-62.

[30] Renlund N, O'Neill FH, Zhang L, Sidis Y, Teixeira J. Activin receptor-like kinase-2 inhibits activin signaling by blocking the binding of activin to its type II receptor. J Endocrinol 2007; 195: 95-103.

[31] Rigueur D, Brugger S, Anbarchian T, Kim JK, Lee Y, Lyons KM. The Type I BMP Receptor ACVR1/ALK2 is Required for Chondrogenesis During Development. J Bone Min Res 2015; 30: 733-41.

[32] Lin S, Svoboda KK, Feng JQ, Jiang X. The biological function of type I receptors of bone morphogenetic protein in bone. Bone Res 2016; 4: 16005.

[33] Pacifici M, Shore EM. Common mutations in ALK2/ACVR1, a multi-faceted receptor, have roles in distinct pediatric musculoskeletal and neural orphan disorders. Cytokine Growth Factor Rev 2016; 27: 93-104.

[34] Ardelean DS, Jerkic M, Yin M, Peter M, Ngan B, Kerbel RS, Foster FS, Letarte M. Endoglin and activin receptor-like kinase 1 heterozygous mice have a distinct pulmonary and hepatic angiogenic profile and response to anti-VEGF treatment. Angiogenesis 2013; 17: 129-46.

[35] Bagarova J, Vonner AJ, Armstrong KA, Borgermann J, Lai CS, Deng DY, Beppu H, Alfano I, Filippakopoulos P, Morrell NW, Bullock AN, Knaus P, Mishina Y, Yu PB. Constitutively active ALK2 receptor mutants require type II receptor cooperation. Mol Cell Biol 2013; 33: 2413-24.

[36] Buczkowicz P, Hoeman C, Rakopoulos P, Pajovic S, Letourneau L, Dzamba M, Morrison A, Lewis P, Bouffet E, Bartels U, Zuccaro J, Agnihotri S, Ryall S, Barszczyk M, Chornenkyy Y, Bourgey M, Bourque G, Montpetit A, Cordero F, Castelo-Branco P, Mangerel J, Tabori U, Ho KC, Huang A, Taylor KR, Mackay A, Bendel AE, Nazarian J, Fangusaro JR, Karajannis MA, Zagzag D, Foreman NK, Donson A, Hegert JV, Smith A, Chan J, Lafay-Cousin L, Dunn S, Hukin J, Dunham C, Scheinemann K, Michaud J, Zelcer S, Ramsay D, Cain J, Brennan C, Souweidane MM, Jones C, Allis CD, Brudno M, Becher O, Hawkins C. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. Nat Genet 2014; 46: 451-6.

[37] Herrera B, van Dinther M, Dijke ten P, Inman GJ. Autocrine Bone Morphogenetic Protein-9 Signals through Activin Receptor-like Kinase-2/Smad1/Smad4 to Promote Ovarian Cancer Cell Proliferation. Cancer Res 2009; 69: 9254-62.

[38] Olsen OE, Wader KF, Misund K, Vatsveen TK, Ro TB, Mylin AK, Turesson I, Stordal BF, Moen SH, Standal T, Waage A, Sundan A, Holien T. Bone morphogenetic protein-9 suppresses growth of myeloma cells by signaling through ALK2 but is inhibited by endoglin. Blood Cancer J 2014; 4: e196-8.

[39] Na YR, Seok SH, Kim DJ, Han JH, Kim TH, Jung H, Lee BH, Park JH. Bone morphogenetic protein 7 induces mesenchymal-to-epithelial transition in melanoma cells, leading to inhibition of metastasis. Cancer Sci 2009; 100: 2218-25.

[40] Hao J, Ho JN, Lewis JA, Karim KA, Daniels RN, Gentry PR, Hopkins CR, Lindsley CW, Hong CC. In Vivo Structure-Activity Relationship Study of Dorsomorphin Analogues Identifies Selective VEGF and BMP Inhibitors. ACS Chem Biol 2010; 5: 245-53.

[41] Neely MD, Litt MJ, Tidball AM, Li GG, Aboud AA, Hopkins CR, Chamberlin R, Hong CC, Ess KC, Bowman AB. DMH1, a Highly Selective Small Molecule BMP Inhibitor Promotes Neurogenesis of hiPSCs: Comparison of PAX6 and SOX1 Expression during Neural Induction. ACS Chem Neurosci 2012; 3: 482-91.

[42] Bowman TV, Zon LI. Swimming into the Future of Drug Discovery: In Vivo Chemical Screens in Zebrafish. ACS Chem Biol 2010; 5: 159-61.

[43] 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.

[44] Kerr G, Sheldon H, Chaikuad A, Alfano I, Delft von F, Bullock AN, Harris AL. A small molecule targeting ALK1 prevents Notch cooperativity

and inhibits functional angiogenesis. Angiogenesis 2015; 18: 209-17.
[45] Lee HJ, Kim PH. Further Characterization of Activin A-induced IgA Response in Murine B Lymphocytes. Immune Netw 2009; 9: 133.
[46] Wakefield LM, Hill CS. Beyond TGFβ: roles of other TGFβ superfamily members in cancer. Nature Rev Cancer 2013; 13: 328-41.
[47] Mishina Y, Suzuki A, Ueno N, Behringer RR. Bmpr encodes a type I bone porphogenetic protein receptor that is essential for gastrulation during mouse embryongenesis. Genes Dev 1995; 9: 3027-37.
[48] Jing J, Ren Y, Zong Z, Liu C, Kamiya N, Mishina Y, Liu Y, Zhou X, Feng JQ. BMP Receptor 1A Determines the Cell Fate of the Postnatal Growth Plate. Int J Biol Sci 2013; 9: 895-906.
[49] Kamiya N, Ye L, Kobayashi T, Lucas DJ, Mochida Y, Yamauchi M, Kronenberg HM, Feng JQ, Mishina Y. Disruption of BMP Signaling in Osteoblasts Through Type IA Receptor (BMPRIA) Increases Bone Mass. J Bone Min Res 2008; 23: 2007-17.
[50] Schille C, Heller J, Schambony A. Differential requirement of bone morphogenetic protein receptors Ia (ALK3) and Ib (ALK6) in early embryonic patterning and neural crest development. BMC Dev Biol 2016; 16: 1.
[51] Bhattacharya S, MacDonald ST, Farthing CR. Molecular mechanisms controlling the coupled development of myocardium and coronary vasculature. Clin Sci 2006; 111: 35-46.
[52] Lockhart MM, Boukens BJ, Phelps AL, Brown CL, Toomer KA, Burns TA, Mukherjee RD, Norris RA, Trusk TC, van den Hoff MJ, Wessels A. Alk3 mediated Bmp signaling controls the contribution of epicardially derived cells to the tissues of the atrioventricular junction. Dev Biol 2014; 396: 8-18.
[53] Maloum F, Allaire JM, Gagne-Sansfacon J, Roy E, Belleville K, Sarret P, Morrisset J, Carrier JC, Mishina Y, Kaestner KH, Perreault N. Epithelial BMP signaling is required for proper specification of epithelial cell lineages and gastric endocrine cells. Am J Physiol Liver Physiol 2011; 300: G1065-79.
[54] Howe JR, Bair JL, Sayed MG, Anderson ME, Mitros FA, Petersen GM, Velculescu VE, Traverso G, Vogelstein B. Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. Nat Genet 2001; 28: 184-7.
[55] Howe JR, Sayed MG, Ahmed AF, Ringold J, Larsen-Haidle J, Merg A, Mitros FA, Vaccaro CA, Petersen GM, Giardiello FM, Tinley ST, Aaltonen LA. The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations. J Med Genet 2004; 41: 484-91.
[56] Zhou XP, Woodford-Richens K, Lehtonen R, Kurose K, Aldred M, Hampel H, Launonen V, Virta S, Pilarski R, Salovaara R, Bodmer WF, Conrad BA, Dunlop M, Hodgson SV, Iwama T, Järvinen H, Kellokumpu I, Kim JC, Leggett B, Markie D, Mecklin JP, Neale K, Phillips R, Piris J, Rozen P, Houlston RS, Aaltonen LA, Tomlinson IP, Eng C. Germline Mutations in BMPR1A/ALK3 Cause a Subset of Cases of Juvenile Polyposis Syndrome and of Cowden and Bannayan-Riley-Rvalcaba Syndrome. Am J Hum Genet 2001; 69: 704-11.
[57] Kim IJ, Park JH, Kang HC, Kim KH, Kim JH, Ku JL, Kang SB, Park SY, Lee JS, Park JG. Identification of a novel BMPR1A germline mutation in a Korean juvenile polyposis patient without SMAD4 mutation. Clin Genet 2003; 63: 126-30.
[58] Wirtzfeld DA, Petrelli NJ, Rodriguez-Bigas MA. Hamartomatous Polyposis Syndromes: Molecular Genetics, Neoplastic Risk, and Surveillance Recommendations. Ann Surg Oncol 2001; 8: 319-27.
[59] Aaltonen L, Johns L, Jarvinen H, Mecklin JP, Houlston R. Explaining the Familial Colorectal Cancer Risk Associated with Mismatch Repair (MMR)-Deficient and MMR-Stable Tumors. Clin Cancer Res 2007; 13: 356-61.
[60] Chang YC, Chang JG, Liu TC, Lin CY, Yang SF, Ho CM, Chen WT, Change YS. Mutation analysis of 13 driver genes of colorectal cancer-related pathways in Taiwanese patients. World J Gastroenterol 2016; 22: 2314-25.
[61] Voorneveld PW, Stache V, Jacobs RJ, Smolders E, Sitters AI, Liesker A, Korkmaz KS, Lam SM, De Miranda NF, Morreau H, Kodach LL, Hardwick JC. Reduced expression of bone morphogenetic protein receptor IA in pancreatic canceris associated with a poor prognosis. Br J Cancer 2013; 109: 1805-12.
[62] Pickup MW, Hover LD, Guo Y, Gorzka AE, Chytil A, Novitskiy SV, Moses HL, Owens P. Deletion of the BMP receptor BMPR1a impairs mammary tumor formation and metastasis. Oncotarget 2015; 6: 22890-904.
[63] O'Neill HL, Cassidy AP, Harris OB, Cassidy JW. BMP2/BMPR1A is linked to tumour progression in dedifferentiated liposarcomas. PeerJ 2016; 4: e1957.
[64] Tsugawa D, Oya Y, Masuzaki R, Ray K, Engers DW, Dib M, Do N, Kuramitu K, Ho K, Frist A, Yu PPB, Bloch KD, Lindsley CW, Hopkins CR, Hong CC, Karp SJ. Specific Activin Receptor-Like Kinase 3 Inhibitors Enhance Liver Regeneration. J Pharmacol Exp Ther 2014; 351: 549-58.
[65] Chen Y, Mironova E, Whitaker LL, Edwards L, Yost HJ, Ramsdell AF. ALK4 functions as a receptor for multiple TGFβ-related ligands to regulate left-right axis determination and meso-

derm induction in Xenopus. Dev Biol 2004; 268: 280-94.
[66] Armes NA, Smith JC. The ALK-2 and ALK-4 activin receptors transduce distinct mesoderm-inducing signals during early Xenopus development but do not co-operate to establish thresholds. Development 1997; 124: 3797-804.
[67] Gu Z, Nomura M, Simpson BB, Lei H, Feijen A, van den Eijnden-van Raaij J, Donahue PK. The type I activin receptor ActRIB is required for egg cylinder organization and gastrulation in the mouse. Genes Dev 1998; 12: 844-57.
[68] Siragam V, Jeyapalan Rutnam Z, Yang W, Fang L, Luo L, Yang X, Li M, Deng Z, Qian J, Peng C, Yanag BB. MicroRNA miR-98 inhibits tumor angiogenesis and invasion by targeting activin receptor-like kinase-4 and matrix metalloproteinase-11. Oncotarget 2012; 3: 1370-85.
[69] Li Y, Klxausen C, Zhu H, Leung PC. Activin A Increases Human Trophoblast Invasion by Inducing SNAIL-Mediated MMP2 Up-Regulation Through ALK4. J Clin Endocrinol Metab 2015; 100: E1415-27.
[70] Peng J, Fullerton PT Jr, Monsivais D, Clementi C, Su GH, Matzuk MM. Uterine Activin-Like Kinase 4 Regulates Trophoblast Development During Mouse Placentation. Mol Endocrinol 2015; 29: 1684-93.
[71] Miles DC, Wakeling SI, Stringer JM, van den Bergen JA, Wilhelm D, Sinclair AH, Western PS. Signaling through the TGFβ-Activin Receptors ALK4/5/7 Regulates Testis Formation and Male Germ Cell Development. PLoS One 2013; 8: e54606.
[72] Klauzinska M, Castro NP, Rangel MC, Spike BT, Gray PC, Bertolette D, Cuttitta F, Salomon D. The multifaceted role of the embryonic gene Cripto-1 in cancer, stem cells and epithelial-mesenchymal transition. Sem Cancer Biol 2014; 29: 51-8.
[73] Law J, Zhang G, Dragan M, Postovit LM, Bhattacharya M. Cellular Signaling. Cell Signal 2014; 26: 1935-42.
[74] Qiu W, Li X, Tang H, Huang AS, Panteleyev AA, Owens DM, Su GH. Conditional Activin Receptor Type 1B (Acvr1b) Knockout Mice Reveal Hair Loss Abnormality. J Invest Dermatol 2010; 131: 1067-76.
[75] Ripoche D, Gout J, Pommier RM, Jaafar R, Zhang CX, Bartholin L, Bertolino P. Generation of a conditional mouse model to target Acvr1b disruption in adult tissues. Genesis 2013; 51: 120-7.
[76] Alexander JM, Bikkal HA, Zervas NT, Laws ER, Klibanski A. Tumor-Specific Expression and Alternate Splicing of Messenger Ribonucleic Acid Encoding Activin/Transforming growth Factor-β Receptors in Human Pituitary Adenomas. J Clin Endocrinol Metab 1996; 81: 783-90.
[77] Danila DC, Zhang X, Zhou Y, Haidar JN, Klibanski A. Overexpression of Wild-Type Activin Receptor Alk4-1 Restores Activin Antiproliferative Effects in Human Pituitary Tumor Cells. J Clin Endocrinol Metab 2002; 87: 4741-6.
[78] Su GH, Bansal R, Murphy KM, Montgomery E, Yeo CJ, Hruban RH, Kern SE. ACVR1B (ALK4, activin receptor type 1B) gene mutations in pancreatic carcinoma. Proc Natl Acad Sci U S A 2001; 98: 3254-7.
[79] Lonardo E, Frias-Aldeguer J, Hermann PC, Heeschen C. Pancreatic stellate cells form a niche for cancer stem cells and promote their self-renewal and invasiveness. Cell Cycle 2014; 11: 1282-90.
[80] Vo BT, Khan SA. Expression of nodal and nodal receptors in prostate stem cells and prostate cancer cells: Autocrine effects on cell proliferation and migration. Prostate 2011; 71: 1084-96.
[81] Dias V, Meachem S, Rajpert-De Meyts E, McLachlan R, Manuelpillai U, Loveland KL. Activin receptor subunits in normal and dysfunctional adult human testis. Hum Reprod 2007; 23: 412-20.
[82] Landis MD, Seachrist DD, Montanez-Wiscovich ME, Danielpour D, Kerl RA. Gene expression profiling of cancer progression reveals intrinsic regulation of transforming growth factor-β signaling in ErbB2/Neu-induced tumors from transgenic mice. Oncogene 2005; 24: 5173-90.
[83] Murakami M, Suzuki M, Nishino Y, Funaba M. Regulatory expression of genes related to metastasis by TGF-β and activin A in B16 murine melanoma cells. Mol Biol Rep 2009; 37: 1279-86.
[84] DaCosta Byfield S, Major C, Laping NJ, Roberts AB. SB-505124 Is a Selective Inhibitor of Transforming Growth Factor-β Type I Receptors ALK4, ALK5, and ALK7. Mol Pharmacol 2004; 65: 744-52.
[85] Inman GJ, Nicolás FJ, Callahan JF, Harling JD, Gaster LM, Reith AD, Laping NJ, Hill CS. SB-431542 is a potent and specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. Mol Pharmacol 2002; 62: 65-74.
[86] Fields SZ, Parshad S, Anne M, Raftopoulos H, Alexander MJ, Sherman ML, Laadem A, Sung V, Terpos E. Activin receptor antagonists for cancer-related anemia and bone disease. Expert Opin Investig Drugs 2013; 22: 87-101.
[87] Fang L, Chang HM, Cheng JC, Yu Y, Leung PC, Sun YP. Growth Differentiation Factor-8 Decreases StAR Expression Through ALK5-Medi-

ated Smad3 and ERK1/2 Signaling Pathways in Luteinized Human Granulosa Cells. Endocrinology 2015; 156: 4684-94.
[88] Wang Y, Nicholls PK, Stanton PG, Harrison CA, Sarraj M, Gilchrist RB, Findlay JK, Farnworth PG. Extra-ovarian expression and activity of growth differentiation factor 9. J Endocrinol 2009; 202: 419-30.
[89] Larsson J, Goumans MJ, Jansson Sjostrand L, van Rooijen MA, Ward D, Leveen P, Xu X, ten Dijke P, Mummery CL, Karlsson S. Abnormal angiogenesis but intact hematopoietic potential in TGFβ type I receptor-deficient mice. EMBO J 2001; 20: 1663-73.
[90] Itoh F, Itoh S, Carvalho RL, Adachi T, Ema M, Goumans MJ, Larsson J, Karlsson S, Takahashi S, Mummery CL, ten Dijke P, Kato M. Poor vessel formation in embryos from knock-in mice expressing ALK5 with L45 loop mutation defective in Smad activation. Lab Invest 2009; 89: 800-10.
[91] Li WY, Dudas M, Kaartinen V. Signaling through Tgf-β type I receptor Alk5 is required for upper lip fusion. Mech Dev 2008; 125: 874-82.
[92] Sridurongrit S, Larsson J, Schwartz R, Ruiz-Lozano P, Kaartinen V. Signaling via the Tgf-β type I receptor Alk5 in heart development. Dev Biol 2008; 322: 208-18.
[93] James JM, Nalbandian A, Mukouyama YS. TGFβ signaling is required for sprouting lymphangiogenesis during lymphatic network development in the skin. Development 2013; 140: 3903-14.
[94] Gao Y, Li S, Li Q. Uterine epithelial cell proliferation and endometrial hyperplasia: evidence from a mouse model. Mol Hum Reprod 2014; 20: 776-86.
[95] Peng J, Monsivais D, You R, Zhong H, Pangas SA, Matzuk MM. Uterine activin receptor-like kinase 5 is crucial for blastocyst implantation and placental development. Proc Natl Acad Sci U S A 2015; 112: E5098-107.
[96] Gao Y, Duran S, Lydon JP, DeMayo FJ, Burghardt RC, Bayless KJ, Bartholin L, Li Q. Constitutive Activation of Transforming Growth Factor Beta Receptor 1 in the Mouse Uterus Impairs Uterine Morphology and Function. Biol Reprod 2015; 92: 34-4.
[97] Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamuire Y, White R, Smits AMM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med 1988; 319: 525-32.
[98] Pasche B, Pennison MJ, Jimenez H, Wang M. TGFBR1 and Cancer Susceptibility. Trans Am Clin Climatol Soc 2014; 125: 300-12.
[99] Pasche B, Knobloch TJ, Bian Y, Liu J, Phukan S, Rosman D, Kakalamani V, Baddi L, Siddiqui FS, Frankel W, Prior TW, Schuller DE, Agrawal A, Lang J, Dolan ME, Vokes EE, Lane WS, Huang CC, Caldes T, Di Cristofano A, Hampel H, Nilsson I, von Heijne G, Fodde R, Murty VV, de la Chapelle A, Weghorst CM. Somatic Acquisition and Signaling of TGFBR1*6A in Cancer. JAMA 2005; 294: 1634-46.
[100] Zeng Q, Phukan S, Xu Y, Sadim M, Rosman DS, Pennison M, Liao J, Yang GY, Huang CC, Valle L, Di Cristofano A, de la Chapelle A, Pasche B. Tgfbr1 Haploinsufficiency Is a Potent Modifier of Colorectal Cancer Development. Cancer Res 2009; 69: 678-86.
[101] Lui VWY, Hedberg ML, Li H, Vangara BS, Pendleton K, Zeng Y, Lu Y, Zhang Q, Du Y, Gilbert BR, Freilino M, Sauerwein S, Peyser ND, Xiao D, Diergaarde B, Wang L, Chiosea S, Seethala R, Johnson JT, Kim S, Duvvuri U, Ferris RL, Romkes M, Nukui T, Kwok-Shing Ng P, Garraway LA, Hammerman PS, Mills GB, Grandis JR. Frequent Mutation of the PI3K Pathway in Head and Neck Cancer Defines Predictive Biomarkers. Cancer Discov 2013; 3: 761-9.
[102] Squarize CH, Castilho RM, Abrahao AC, Molinolo A, Lingen MW, Gutkind JS. PTEN Deficiency Contributes to the Development and Progression of Head and Neck Cancer. Neoplasia 2013; 15: 461-71.
[103] Bian Y, Hall B, Sun ZJ, Molinolo A, Chen W, Gutkind JS, Waes CV, Kulkarni AB. Loss of TGF-β signaling and PTEN promotes head and neck squamous cell carcinoma through cellular senescence evasion and cancer-related inflammation. Oncogene 2011; 31: 3322-32.
[104] Adrian K, Strouch MJ, Zeng Q, Barron MR, Cheon EC, Honasoge A, Xu Y, Phukan S, Sadim M, Bentrem DJ, Pasche B, Grippo PJ. Tgfbr1 Haploinsufficiency Inhibits the Development of Murine Mutant Kras-Induced Pancreatic Precancer. Cancer Res 2009; 69: 9169-74.
[105] Matsuyama S, Iwadate M, Kondo M, Saitoh M, Hanyu A, Shimizu K, Aburatani H, Mishima HK, Imamura T, Miyazono K, Miyazawa K. SB-431542 and Gleevec Inhibit Transforming Growth Factor-Beta-Induced Proliferation of Human Osteosarcoma Cells. Cancer Res 2003; 63: 7791-8.
[106] Gao Y, Vincent DF, Davis AJ, Sansom OJ, Bartholin L, Li Q. Constitutively active transforming growth factor β receptor 1 in the mouse ovary promotes tumorigenesis. Oncotarget 2016; 7: 40904-18.
[107] Jin CH, Krishnaiah M, Sreenu D, Subrahmanyam VB, Rao KS, Lee HJ, Park SJ, Park HJ, Lee K, Sheen YY, Kim DK. Discovery of N-((4-([1,2,4]Triazolo[1,5- a]pyridin-6-yl)-5-(6-methylpyridin-2-yl)-1 H-imidazol-2-yl)methyl)-2-fluoroaniline (EW-7197): A Highly Potent, Selective, and Orally Bioavailable Inhibitor of TGF-β Type

I Receptor Kinase as Cancer Immunotherapeutic/Antifibrotic Agent. J Med Chem 2014; 57: 4213-38.
[108] Bartscht T, Rosien B, Rades D, Kaufmann R, Biersack H, Lehnert H, Gieseler F, Ungefroren H. Dasatinib blocks transcriptional and promigratory responses to transforming growth factor-beta in pancreatic adenocarcinoma cells through inhibition of Smad signaling: implications for in vivo mode of action. Mol Cancer 2015; 14: 199.
[109] Yoon JH, Jung SM, Park SH, Kato M, Yamashita T, Lee IK, Sudo K, Nakae S, Han JS, Kim OH, Oh BC, Sumida T, Kuroda M, Ju JH, Jung KC, Park SH, Kim DK, Mamura M. Activin receptor-like kinase5 inhibition suppresses mouse melanoma by ubiquitin degradation of Smad4, thereby derepressing eomesodermin in cytotoxic T lymphocytes. EMBO Mol Med 2013; 5: 1720-39.
[110] Basal E, Ayeni T, Zhang Q, Langstraat C, Donahue PK, Pepin D, Yin X, Leo E, Cliby W. Patterns of Mullerian inhibiting substance type II and candidate type I receptors in epithelial ovarian cancer. Curr Mol Med 2016; 16: 222-31.
[111] Stange K, Désir J, Kakar N, Mueller TD, Budde BS, Gordon CT, Horn D, Seemann P, Borck G. A hypomorphic BMPR1B mutation causes du Pan acromesomelic dysplasia. Orphanet J Rare Dis 2015: 1-6.
[112] Seeman P, Schwappacher R, Kjaer KW, Krakow D, Lehmann K, Dawson K, Stricker S, Pohl J, Ploger F, Staub E, Nickel J, Sebald W, Knaus P, Mundlos S. Activating and deactivating mutations in the receptor interaction site of GDF5 cause symphalangism or brachydactyly type A2. J Clin Invest 2005; 115: 2373-81.
[113] Racacho L, Byrnes AM, MacDonald H, Dranse HJ, Nikkel SM, Allanson J, Rosser E, Underhill TM, Bulman DE. Two novel disease-causing variants in BMPR1B are associated with brachydactyly type A1. Eur J Hum Genet 2015; 23: 1640-5.
[114] Beighton P. Heterozygous manifestations in the heritable disorders of the skeleton. Ped Radiol 1997; 27: 397-401.
[115] Graul-Neumann LM, Deichsel A, Wille U, Kakar N, Koll R, Bassir C, Ahmad J, Cormier-Daire V, Mundlos S, Kubisch C, Borck G, Klopocki E, Mueller TD, Doelken SC, Seemann P. Homozygous missense and nonsense mutations in BMPR1B cause acromesomelic chondrodysplasia-type Grebe. Eur J Hum Genet 2013; 22: 726-33.
[116] Regan SLP, Knight PG, Yovich JL, Stanger JD, Leung Y, Arfuso F, Dharmarajan A, Almahbobi G. Dysregulation of granulosal bone morphogenetic protein receptor 1B density is associated with reduced ovarian reserve and the age-related decline in human fertility. Mol Cell Endocrinol 2016; 425: 84-93.
[117] Regan SL, McFarlane JR, O'Shea T, Andronicos N, Arfuso F, Dharmarajan A, Almahbobi G. Flow cytometric analysis of FSHR, BMRR1B, LHR and apoptosis in granulosa cells and ovulation rate in merino sheep. Reproduction 2015; 150: 151-63.
[118] Laperrousaz B, Jeanpierre S, Sagomy K, Voeltzel T, Ramas S, Kaniewski B, Ffrench M, Salesse S, Nicolini FE, Maguer-Satta V. Primitive CML cell expansion relies on abnormal levels of BMPs provided by the niche and on BMPR1b overexpression. Blood 2013; 122: 3767-77.
[119] Chapellier M, Maguer-Satta V. BMP2, a key to uncover luminal breast cancer origin linked to pollutant effects on epithelial stem cells niche. Mol Cell Oncol 2016; 3: e1026527.
[120] Chapellier M, Bachelard-Cascales E, Schmidt X, Clément F, Treilleux I, Delay E, Jammot A, Menetrier-Caux C, Pochon G, Besancon R, Voeltzel T, de Fromentel CC, Caux C, Blay JY, Iggo R, Maguer-Satta V. Disequilibrium of BMP2 levels in the breast stem cell niche launches epithelial transformation by overamplifying BMPR1B cell response. Stem Cell Rep 2015; 4: 239-54.
[121] Voorneveld PW, Kodach LL, Jacobs RJ, Liv N, Zonnevylle AC, Hoogenboom JP, Biemond I, Verspaget HW, Hommes DW, de Rooij K, van Noesel CJ, Morreau H, van Wezel T, Offerhaus GJ, van den Brink GR, Peppelenbosch MP, ten Dijke P, Hardwick JC. Loss of SMAD4 Alters BMP Signaling to Promote Colorectal Cancer Cell Metastasis via Activation of Rho and ROCK. Gastroenterology 2014; 147: 196-208. e13.
[122] Liu S, Yin F, Fan W, Wang S, Guo XR, Zhang JN, Tian NM, Fan M. Over-expression of BMPR-IB reduces the malignancy of glioblastoma cells by upregulation of p21 and p27Kip1. J Exp Clin Cancer Res 2012; 31: 52.
[123] Gonzalez-Gomez P, Crecente-Campo J, Zahonero C, la Fuente de M, Hernandez-Lain A, Mira H, Sanchez-Gomez P, Garcia-Fuentes M. Controlled release microspheres loaded with BMP7 suppress primary tumors from human glioblastoma. Oncotarget 2015; 6: 10950-63.
[124] Shelton JR, Balzarini J, Peterson MA. Discovery of a nanomolar inhibitor of lung adenocarcinoma in vitro. Bioorg Med Chem 2014; 24: 5107-10.
[125] Takahashi M, Otsuka F, Miyoshi T, Otani H, Goto J, Yamashita M, Ogura T, Makino H, Doihara H. Bone morphogenetic protein 6 (BMP6) and BMP7 inhibit estrogen-induced proliferation of breast cancer cells by suppressing p38 mitogen-activated protein kinase activation. J Endocrinol 2008; 199: 445-55.

[126] Yan K, Wu Q, Yan DH, Lee CH, Rahim N, Tritschler I, DeVecchio J, Kalady MF, Hjelmeland AB, Rich JN. Glioma cancer stem cells secrete Gremlin1 to promote their maintenance within the tumor hierarchy. Genes Dev 2014; 28: 1085-100.
[127] Jornvall H, Blokzijl A, Dijke PT, Ibanez CF. The Orphan Receptor Serine/Threonine Kinase ALK7 Signals Arrest of Proliferation and Morphological Differentiation in a Neuronal Cell Line. J Biol Chem 2001; 276: 5140-6.
[128] Andersson O, Korach-Andre M, Reissman E, Ibanez CF, Bertolino P. Growth/differentiation factor 3 signals through ALK7and regulates accumulation of adipose tissue and diet-induced obesity. Proc Natl Acad Sci U S A 2008; 105: 7252-6.
[129] Ying S, Cao H, Hu H, Wang X, Tang Y, Huang C. Alk7 Depleted Mice Exhibit Prolonged Cardiac Repolarization and Are Predisposed to Ventricular Arrhythmia. PLoS One 2016; 11: e0149205.
[130] Khalil AM, Dotimas H, Kahn J, Lamerdin JE, Hayes DB, Gupta P, Franti M. Differential Binding Activity of TGF-β Family Proteins to Select TGF-β Receptors. J Pharmacol Exp Ther 2016; 358: 423-30.
[131] Reissman E, Jornvall H, Blokzijl A, Andersson O, Chang C, Minchiotti G, Persico MG, Ibanez CF. The orphan receptor ALK7 and the Activin receptor ALK4 mediate signaling by Nodal proteins during vertebrate development. Genes Dev 2001; 15: 2010-22.
[132] Li J, Yang Z, Zou Q, Yuan Y, Li J, Liang L, Zeng G, Chen S. PKM2 and ACVR 1C are prognostic markers for poor prognosis of gallbladder cancer. Clin Transl Oncol 2013; 16: 200-7.
[133] Zeng F, Xu G, Zhou T, Yang C, Wang X, Peng C, Zhou H. Reduced expression of activin receptor-like kinase 7 in breast cancer is associated with tumor progression. Med Oncol 2012; 29: 2519-26.
[134] Xu G, Zhou H, Wang Q, Auersperg N, Peng C. Activin Receptor-Like Kinase 7 Induces Apoptosis through Up-Regulation of Bax and Down-Regulation of Xiap in Normal and Malignant Ovarian Epithelial Cell Lines. Mol Cancer Res 2006; 4: 235-46.
[135] Ye G, Fu G, Cui S, Zhao S, Bernaudo S, Bai Y, Ding Y, Zhang Y, Yang BB, Peng C. MicroRNA 376c enhances ovarian cancer cell survival by targeting activin receptor-like kinase 7: implications for chemoresistance. J Cell Sci 2011; 124: 359-68.
[136] Nam JS, Terabe M, Mamura M, Kang MJ, Chae H, Stuelten C, Kohn E, Tang B, Sabzevari H, Anver MR, Lawrence S, Danielpour D, Lonning S, Berzofsky JA, Wakefield LM. An Anti-Transforming Growth Factor Beta Antibody Suppresses Metastasis via Cooperative Effects on Multiple Cell Compartments. Cancer Res 2008; 68: 3835-43.