ALK1 as an emerging target for antiangiogenic therapy of cancer
Sara I. Cunha1 and Kristian Pietras1

Members of the TGF-β family act on many, if not all, cell types within the body, producing diverse and complex cellular outcomes. Activation of the endothelial cell-restricted TGF-β type I receptor ALK1 results from the binding of several different ligands of the TGF-β family, including bone morphogenetic protein (BMP) 9, BMP10, and TGF-β. Mounting genetic, pharmacologic, and histopathologic evidence supports a critical role for ALK1 signaling in regulation of both developmental and pathologic blood vessel formation. However, the precise function of TGF-β family signaling in endothelial cells is difficult to predict and appears highly context dependent because of the multitude of ligands and receptors influencing the final outcome. Pharmacologic inhibitors of ALK1 have recently been developed and will allow for more accurate studies of ALK1 function in vivo, as well as for assessment of ALK1 as a target for suppression of angiogenesis during tumor development. Herein, we will summarize the current view of ALK1 regulation of endothelial cell phenotype in vitro and in vivo as well as provide an outlook for the ongoing clinical trials of ALK1 inhibitors in malignant disease. (Blood. 2011; 117(26):6999-7006)

Introduction

The introduction of antiangiogenic therapies into oncologic practice has been highly anticipated because of the success of extensive preclinical testing. However, the initial clinical experience with this new class of anticancer drugs has been sobering, with a measurable therapeutic benefit of a few months observed and very little effect on overall patient survival. To fully realize the potential of therapies inhibiting neoangiogenesis, it is likely that drugs impinging on multiple regulatory pathways must be combined, and thus we must learn more about the biology of various signaling factors affecting endothelial cell (EC) growth and function. Activin receptor-like kinase 1 (ALK1) is an EC-restricted receptor of the large TGF-β family.1 Herein, we will review our current understanding of ALK1 signaling and the potential of ALK1 to serve as a drug target for antiangiogenic therapy for cancer.

Signaling by ALK1 in ECs

The large family of TGF-β extracellular ligands consists of > 30 cytokines that exert influence on several cellular compartments, notably epithelial cells, fibroblasts, immune cells, and endothelial and perivascular cells. TGF-β, the prototypic member of the family, elicits a diverse set of cellular responses, such as growth arrest, immune suppression, differentiation, apoptosis, and specification of developmental cell fate during embryogenesis and pathogenesis, in species ranging from flies and worms to mammals.2,3 On secretion and subsequent activation, the mature TGF-β ligand initiates signaling by inducing specific serine/threonine kinase type I and type II receptor heterotetrameric complexes.4 Ligand binding results in signal propagation inside the cell by phosphorylation of specific effector proteins, so-called Smads, which translocate to the nucleus and activate transcription of target genes.2,5 In ECs, TGF-β has been shown to signal via both the ubiquitously expressed TGF-β type I receptor ALK5 and through the predominantly EC restricted receptor ALK1 (Figure 1). Depending on which type I receptor is recruited, different Smad signaling cascades are activated; ALK1 activation induces phosphorylation of Smad1/5/8, whereas ALK5 leads to Smad 2/3 activation.6-9 Consequent to engagement of either Smad pathway, the receptor-activated Smads further form a heteromeric complex with a common and related partner molecule, Smad4, which translocates the complexes into the nucleus, where cell type-specific transcriptional modulators collaborate to activate or repress transcription of specific target genes in the angiogenic response.10,11 In addition to the canonical signaling through Smad activation, TGF-β stimulation may lead to Smad-independent regulation of cellular outcomes, such as apoptosis and cell-cycle progression, through the direct modulation of prototypical signaling mediators, including MAP kinases and p21.12

Furthermore, a third type of TGF-β receptor, the type III receptors, is represented by betaglycan and endoglin. Endoglin, primarily a vascular marker, is an auxiliary receptor for TGF-β signaling required for angiogenesis during development and increasingly expressed during EC activation, inflammation, and tumor angiogenesis (Figure 1).13-15 There is no enzymatic kinase activity associated with endoglin, but possibly through presenting various ligands to the receptors, endoglin modulates efficient TGF-β/ALK1 signaling but not TGF-β/ALK5 signaling (see “Related signaling pathways” and Lebrin et al16).

Cellular effects of ALK1 signaling in ECs

Effects of TGF-β on ECs in vitro

Several lines of evidence suggest that TGF-β regulates a fine balance between ALK1 and ALK5 signaling in the endothelium.17,18 However, many reports on the action of ALK1 signaling in ECs have revealed paradoxical results, highlighting the pleiotropic effects of TGF-β. For example, the ALK1 signaling pathway, on stimulation with TGF-β, leads to EC proliferation and migration (Figure 2A). Accordingly, ectopic expression of the ALK1 downstream target gene Id1 induces migration and EC tube formation,19...
stimulation. In agreement with these findings, ALK1 signal targeting gene products, such as PAI-1 and PDGF-B, supporting a role of ALK1 in mediating the maturation phase of angiogenesis. Possible inhibition, and cell adhesion, suggesting that rather than an activator, the constitutively active form of ALK1 inhibits proliferation, migration, and tumor angiogenesis in vivo.22,23 Further attempts to dissect the biologic effect of ALK1 activation have shown that infection of ECs with a (FGF)–induced angiogenesis in ex vivo models (Figure 2A).22,23 Although infection of ECs with a BMP9/ALK1-induced phosphorylation of Smad1/5/8 in bovine aortic ECs. In addition, in agreement with the ALK5-independent action of ALK1 is the notion that ALK5 is present in ECs in vivo only at low levels and instead is postulated to be mainly expressed by perivascular cells, thereby regulating angiogenesis through a paracrine mode of action (Figure 2B).29,30

Consistent with these findings, specific deletion of ALK5 in the endothelium does not result in vascular malformations in mice, nor does ALK5 genetic or pharmacologic inhibition in zebrafish. Nonetheless, embryos from knockout mice expressing ALK5 with a L54 loop mutation (a mutation that interferes with downstream Smad2 phosphorylation while preserving the capability of ALK5 to mediate non-Smad signaling and lateral signaling to ALK1)30 partially rescued the earliest vascular defects observed in ALK5 knockout mice.33

Collectively, these reports suggest that ALK5 signaling is indeed relevant for endothelial homeostasis, but the codependency on ALK1 signaling remains unclear, as does the issue whether this function is EC autonomous or involves crosstalk between ECs and mural cells. In addition to being critical to ensure endothelial architecture, ALK1 appears to also modulate smooth muscle cell differentiation and recruitment, as observed by Oh et al, through the analysis of ALK1 knockout mice. Further studies to address ALK1 vs ALK5 signaling in ECs and in perivascular cells are thus highly warranted to clarify and conciliate the seemingly divergent conclusions from previous studies.

**Related signaling pathways**

Both BMP9 and BMP10 have been identified as functional activators of ALK1 in ECs, inducing comparable cellular effects. Nevertheless, BMP10 binds to ALK1 with lower affinity than BMP9 and is mainly expressed in the murine heart. Moreover, BMP10 ablation leads to embryonic lethality because of impaired cardiac growth and function, suggesting that BMP10 action on ALK1 may be cardiac tissue specific. Whether BMP10 mediates vascular effects similar to BMP9 direct remains to be elucidated.

ALK1 shares similar properties in terms of BMP-dependent activation of Smad1/5/8 signaling with the related BMP type I receptors ALK2, ALK3, and ALK6. Ligand specificity has not been

**Effects of BMP9 on ECs in vitro**

Similar to TGF-β, the recently described high-affinity ligand for ALK1, bone morphogenetic protein (BMP) 9,22 has also been reported to have disparate effects on ECs. On the one hand, BMP9 exhibits angiogenic effects, inhibiting fibroblast growth factor (FGF)–induced angiogenesis in ex vivo models (Figure 2A).22,23 On the other, Suzuki et al24 have shown induction of proliferation by BMP9 in multiple types of ECs in vitro and a proangiogenic effect of BMP9 on Matrigel plug vascularization and tumor angiogenesis in a xenograft model of pancreatic cancer (Figure 2A).

In an attempt to further clarify the role of ALK1 signaling in ECs, we recently described a synergistic effect of TGF-β together with BMP9, where the combined action of both ligands improved the EC response to angiogenic stimuli (Figure 2A).25 Although either ligand alone impairs EC functionality in this context, the presence of both ligands primes ECs to an enhanced response to prototypical angiogenic cues, such as VEGF and FGF-2, both in vitro in different cell lines and in vascular in-growth into subcutaneously injected Matrigel plugs in vivo.

From a molecular point of view, double stimulation of ECs with TGF-β and BMP9 induces a substantial up-regulation of ALK5 target gene products, such as PAI-1 and PDGF-B, supporting a previous report on induction of Smad2 phosphorylation on BMP9 stimulation.26 In agreement with these findings, ALK1 signal inhibition proved to interfere not only with its own target genes but also with ALK5 downstream effectors in a tumor model of multistep tumorigenesis. In this context, it is interesting to note that the relative, rather than the absolute, levels of ALK1 and ALK5 signaling may be crucial for proper regulation of gene expression.

**Interdependency of ALK1/ALK5 signaling**

Transcriptional profiling of ECs expressing constitutively active forms of ALK1 or ALK5 demonstrates substantial differences in the molecular output obtained from either signaling pathway. Moreover, the nonoverlapping expression patterns of murine ALK1 and ALK5 in vivo29 suggest divergent roles in vascular development for each of the 2 type I receptors expressed by ECs. Further complexity to this signaling system is added by the observation that although TGF-β/ALK1 signaling appears to directly antagonize TGF-β/ALK5 signaling, in some circumstances, the presence of ALK5 is an absolute requirement for efficient TGF-β/ALK1 signaling, as demonstrated by studies in which the authors used ECs deficient for ALK5.30

However, the dependency of ALK1 on ALK5 signaling has been questioned and addressed by several groups. For example, Shao et al31 demonstrated that ALK5 suppression does not interfere with BMP9/ALK1-induced phosphorylation of Smad1/5/8 in bovine aortic ECs. In addition, in agreement with the ALK5-independent action of ALK1 is the notion that ALK5 is present in ECs in vivo only at low levels and instead is postulated to be mainly expressed by perivascular cells, thereby regulating angiogenesis through a paracrine mode of action (Figure 2B).29,32

Consistent with these findings, specific deletion of ALK5 in the endothelium does not result in vascular malformations in mice, nor does ALK5 genetic or pharmacologic inhibition in zebrafish. Nonetheless, embryos from knockin mice expressing ALK5 with a L54 loop mutation (a mutation that interferes with downstream Smad2 phosphorylation while preserving the capability of ALK5 to mediate non-Smad signaling and lateral signaling to ALK1)30 partially rescued the earliest vascular defects observed in ALK5 knockout mice.33

Collectively, these reports suggest that ALK5 signaling is indeed relevant for endothelial homeostasis, but the codependency on ALK1 signaling remains unclear, as does the issue whether this function is EC autonomous or involves crosstalk between ECs and mural cells. In addition to being critical to ensure endothelial architecture, ALK1 appears to also modulate smooth muscle cell differentiation and recruitment, as observed by Oh et al, through the analysis of ALK1 knockout mice. Further studies to address ALK1 vs ALK5 signaling in ECs and in perivascular cells are thus highly warranted to clarify and conciliate the seemingly divergent conclusions from previous studies.

**Related signaling pathways**

Both BMP9 and BMP10 have been identified as functional activators of ALK1 in ECs, inducing comparable cellular effects. Nevertheless, BMP10 binds to ALK1 with lower affinity than BMP9 and is mainly expressed in the murine heart. Moreover, BMP10 ablation leads to embryonic lethality because of impaired cardiac growth and function, suggesting that BMP10 action on ALK1 may be cardiac tissue specific. Whether BMP10 mediates vascular effects similar to BMP9 direct remains to be elucidated.

ALK1 shares similar properties in terms of BMP-dependent activation of Smad1/5/8 signaling with the related BMP type I receptors ALK2, ALK3, and ALK6. Ligand specificity has not been
exhaustively elucidated, and many ligands, including BMP2, BMP4, BMP6, BMP7, BMP9, and BMP10, show a diverse range of effects on ECs, making it difficult to ascertain the net effect of stimulation or inhibition of EC migration, proliferation, and survival is illustrated by images from immunostaining of tumor sections for the vascular marker CD31 (red; images are only representations and are not derived from actual experimental settings). (A) TGF-β signaling through ALK1 predominantly acts to stimulate EC growth and migration. TGF-β signaling through ALK5 predominantly acts to inhibit EC growth and migration. BMP9 stimulation of ALK1 has been reported to be either inhibitory or stimulatory in terms of EC growth and migration. Stimulation of ECs with both TGF-β and BMP9, thus engaging both ALK1 and ALK5 simultaneously, primes ECs to an angiogenic stimulus by prototypical angiogenic inducers such as VEGF. (B) Representation of paracrine interaction between ALK1 expressed by ECs and ALK5 expressed by mural cells. (C) Illustration of the large complexity of BMP ligands and type I receptors that collectively regulate EC functionality in a context-dependent manner.

Of note, ALK2 has been demonstrated to up-regulate ALK1 in ECs in response to high-density lipoproteins, after which ALK1 in turn promotes survival by inducing expression of VEGF-A. ALK1 and ALK2 are also coregulated in aortic ECs by high levels of glucose. Moreover, ALK2 stimulates ectopic endothelial-to-mesenchymal transition as a result of activating mutations in the familial syndrome fibrodysplasia ossificans progressiva or after BMP4 stimulation. In agreement with this finding, ECs conditionally...
Physiologic role of ALK1 in the vasculature

Genetic studies of ALK1 function in vivo

ALK1 expression overlaps with sites of vasculogenesis and angiogenesis in early mouse development. Nevertheless, it has also been described to be present in other cell types, such as hepatic stellate cells and chondrocytes. Rodent ALK1 expression has been appreciated in the brain, lung, kidney, and spleen, being predominantly expressed in developing arterial endothelium, whereas it is almost nonexistent in capillaries. As the mouse develops, ALK1 expression in adult blood vessels is suppressed but induced once again during neangiogenesis in wound healing or tumors.

The importance of TGF-β signaling in angiogenesis and vascular remodeling has been emphasized during the last decades by numerous gene-targeting studies in mice that have shown loss of TGF-β signaling components leading, in most cases, to embryonic lethality because of cardiovascular defects. As a case in point, Acvrl1 (ALK1) knockout mice die at midgestation around embryonic day 11.5 because of severe vascular abnormalities, including excessive capillary fusion, hyperdilation of vessels, and deficiency of differentiation and recruitment of vascular smooth muscle cells. The zebrafish ALK1 mutant, violet beauregarde, also presents with vascular malformations with blood flow constrained to a limited number of hyperproliferative cranial vessels, suggesting that ALK1 possesses an important and evolutionarily preserved function in the vasculature.

Aside from the breakthrough in dissecting the importance of ALK1 on embryonic vascular development, the ALK1-lacZ reporter mice have proven very useful in adulthood to show that ALK1 is fundamental for placentation because ALK1 deficiency results in impairment of umbilical and placental blood vessel formation. The critical relevance of TGF-β signaling in vascular development has been further acknowledged by the identification of mutations in TGF-β receptor genes, ACVRL1 and ENG (endoglin), in familial vascular pathologies. Germline mutations in ACVRL1 or ENG are underlying the development of the human syndrome of hereditary hemorrhagic telangiectasia (HHT), which is characterized by cutaneous telangiectases, increasingly severe nosebleeds, and gastrointestinal hemorrhage. In addition, major arteriovenous malformations occur in lung, liver, spleen, or brain and may cause severe morbidity and mortality. Although ALK1 or endoglin-null mice are embryonic lethal as a result of severe vascular malformations, mice lacking one copy of the gene for either ALK1 or endoglin recapitulate the HHT phenotype with age, making them useful models for the study of this vascular pathology.

EC-specific ALK1 knockout in the mouse through deletion of the ALK1 gene from an Acvrl12loxP allele with the EC-specific L1-Cre line results in postnatal lethality at P5, and mice exhibiting hemorrhaging in the brain, lung, and gastrointestinal tract, mirroring to some extent the full ALK1 knockout phenotype. To evaluate the contribution of ALK1 to vascular homeostasis in adult mice, Park et al globally deleted the ALK1 gene by tamoxifen treatment of 2 months old R26;Flk1-Cre;Acvrl12loxP/loxP mice. One single tamoxifen administration resulted in severe internal hemorrhaging and lethality, suggesting ALK1 relevance for vascular homeostasis in small intestine, lung and uterine vessels.

Other relevant mouse models have been generated for loss-of-function studies of the ALK1 downstream target genes, Id1 and Id3. Although transcription of Id1/Id3 is regulated by many BMP ligands and type I receptors through Smad 1/5, their robust induction by constitutively active ALK1 and by BMP9, as well as the requirement of ALK1 for Id1/Id3 to stimulate EC migration, makes it tempting to speculate on a functional relationship between the effects of ALK1 on ECs and Id1/Id3. However, selective knockdown of Id1 in BM-derived endothelial progenitor cells suppresses angiogenesis and consequently impairs metastatic spread of breast tumors to the lung.

ALK1 signaling in lymphangiogenesis

In addition to the well-established expression of ALK1 in blood ECs, ALK1 was recently also reported to be readily expressed by lymphatic ECs. Indeed, stimulation of lymphatic ECs with BMP9 induces downstream target genes, such as Smad6, and inhibition of ALK1 signaling by the use of an ALK1-Fc fusion protein diminishes lymphatic lymphangiogenesis. Interestingly, lymph vessel development is coordinately and synergistically regulated by ALK1 and VEGF receptor-3 signaling, reminiscent of the crosstalk observed between ALK1/ALK5 and VEGF-receptor signaling in blood vessel angiogenesis.

Targeting ALK1 in vivo

Drugs incorporating inhibitory action against ALK1

Despite the large literature on the diverse effects of signaling emanating from ALK1 in ECs, it is difficult to predict the net
outcome of acute inhibition of ALK1 in a tumor setting. Moreover, the complex ligand/receptor binding specificities within the TGF-β family add further uncertainty to the forecast of therapeutic efficacy and side effect profile of different classes of ALK1 inhibitors currently under development. Small molecules incorporating broad inhibitory action against BMP type I receptor kinase activity, including ALK1, such as dorsomorphin and the structural analog LDN-193189, have recently been developed and shown to block both Smad-dependent and -independent signaling induced by BMPs.72,73

Although such drugs may be very useful and potent in inhibiting BMP type I receptor signaling in a range of diseases and familial syndromes,68 it still remains to determine whether they affect tumor angiogenesis induced by BMPs. In fact, the blockade of zebrafish intersomitic vessel formation observed after treatment with dorsomorphin was determined to be because of off-target effects on VEGF signaling.75 Nevertheless, these recent efforts show that small molecule inhibitors of BMP signaling can be readily identified, and although dorsomorphin appears as a poor candidate for clinical development because of its additional targeting of the tumor suppressor AMP kinase, further development of small molecule compounds with a narrower inhibition profile of BMP type I receptors should be pursued. The use of biologic compounds, such as antibodies or soluble extracellular domains (traps), may seemingly allow for more specific inhibition of ALK1 activity. However, caution is warranted because the extensive crosstalk between different signaling pathways and sharing of receptor complexes may result in undesired off-target effects. Thus, an antibody against the extracellular domain of ALK1 would conceivably block the binding and downstream signaling of all BMPs/TGF-βs that activate ALK1 while preserving the capability of those ligands to bind to related receptors, such as ALK5 or ALK2, on ECs or other cell types. Conversely, an ALK1-Fc fusion protein may neutralize all ligands that bind directly to ALK1 and at the same time block signaling by those ligands through other receptors, thereby incorporating a wider inhibition profile. To date, the development of 3 different biologic ALK1 inhibitors for use in vivo has been reported from pharmaceutical companies. First, researchers at Pfizer are currently conducting phase 1 trials of PF-3446962, a fully human monoclonal antibody against ALK1.76 Second, Nissen et al.77 from Genentech reported on the use of an ALK1-Fc fusion protein (amino acids 23-119 of mouse ALK1) to study the dependence of lymphatic vessel formation on ALK1 signaling in mouse. Third, ACE-041 is a human ALK1-Fc fusion protein currently in clinical development by Acceleron Pharma (mouse counterpart RAP-041, amino acids 22-117 of mouse ALK1).77 Interestingly, ACE-041 only binds and neutralizes BMP9 and BMP10, but not TGF-β.25,71

**Clinical relevance of ALK1 in tumor biology**

**Expression of ALK1 in human tumors**

Little is known about the pattern and extent of ALK1 expression in human normal and malignant tissues, mainly because of a lack of suitable reagents for immunohistochemical analysis. The authors of a preliminary study reported a weak but widespread pattern of expression in the vasculature of normal tissues, including positive staining in lymphatic tissues, lung, intestines and pancreas.79 In a recent study by the same organization, ALK1 was found to be widely present on tumor blood vessels, most notably in lymphomas and cancers of the prostate, skin, thyroid, kidney, ovary, lung, pancreas, and liver.78 In the effort to map the abundance and localization of all human proteins, the public Human Proteome Resource program80 has developed and used an antibody against ALK1 for immunostaining of both normal and neoplastic tissues. In this study, ALK1 exhibited abundant expression in the vasculature and stroma by a high proportion of prostate carcinomas (Figure 3). Intriguingly, expression of TGF-β is correlated to vascular density and survival in prostate cancer, making this tumor type of particular interest for further clinical studies using ALK1 inhibitors.81 Further studies investigating the prognostic significance of ALK1 expression in a wide variety of cancers are, however, still lacking and should be a high priority for future efforts.

**Possible side effects from ALK1 inhibition**

Antiangiogenic therapies, mainly in the form of inhibitors of VEGF signaling, have been in routine clinical use for >5 years for various malignancies. Side effects from inhibiting angiogenesis generally are milder than for conventional cytotoxic drugs and include bleeding, hypertension, fatigue, and nausea. Specifically, given the causal relationship between impaired ALK1 signaling and HHT-related symptoms, inhibition of ALK1 signaling in the
measuring the abundance of ALK1-expressing circulating ECs. 

Sec-}

Importantly, the trial design incorporates both functional readouts for treatments, whereas at advanced stages, TGF-β promotes tumor growth and metastatic spread. Thus, it will prove important to consider different aspects of the biology of TGF-β in the development and use of inhibitors of TGF-β actions for the treatment of cancer because blockade of signaling may lead to divergent effects depending on the stage of the disease.

Similarly, in ECs, the net outcome of signaling from TGF-β family receptors is multifarious and determined by which ligands engage ALK1, ALK5, and other BMP type I receptors, as well as endoglin (Figure 2). Inhibition of the proangiogenic aspects of ALK1/ALK5 downstream signaling must be accompanied by preserved growth-inhibitory capabilities of TGF-β signaling through ALK5 on ECs and malignant cells. In this aspect, both ACE-041 and PF-3446962 are promising agents because they block signaling exclusively by BMP9/BMP10 and ALK1, respectively, and do not directly interfere with ALK5 signaling on other cell types within the tumor. The prospects of developing ALK1 inhibitors in the context of combinatorial treatment regimens with cytotoxic drugs and/or other antiangiogenic agents, including VEGF inhibitors, appear particularly appealing. Despite the challenges of the tremendous complexity of signaling outcomes from the TGF-β receptors in the various cell types of a tumor, the reward for harnessing the TGF-β system for therapeutic purposes may be large. The current preclinical data lend support to the notion of ALK1 being an important target for antiangiogenic therapy of human cancer.

**Acknowledgments**

K.P. is the recipient of a Young Investigator Award from the Swedish Cancer Society.

**Authorship**

Conflict-of-interest disclosure: K.P. is named as an inventor on patents owned by the Ludwig Institute for Cancer Research Ltd and licensed to Acceleron Pharma, which is developing the ALK1 inhibitor ACE-041. S.I.C. declares no competing financial interests. No commercial entities had any influence on the conception of this article.

Correspondence: Kristian Pietras, Department of Medical Biochemistry and Biophysics, Division of Vascular Biology Karolinska Institutet, Scheeles Väg 2, SE-171 77 Stockholm, Sweden; e-mail: Kristian.Pietras@ki.se.

**Clinical trials of ALK1 inhibitors**

Despite the paucity of target expression and validation studies, 2 different clinical phase 1 trials have been launched. First, researchers from Pfizer are testing treatment with PF-3446962 in patients with advanced solid tumors. The primary end point for the study is to recommend a maximum tolerated dose for further phase 2 trials, whereas secondary outcomes include to record preliminary signs of antitumor activity. Importantly, the trial design incorporates both functional readouts for vessel patency by dynamic contrast-enhanced magnetic resonance imaging, as well as a search for surrogate markers of target inhibition by measuring the abundance of ALK1-expressing circulating ECs. 

**Perspective**

Members of the TGF-β family have pleiotropic and complex effects on many, if not all, cell types within the body. Specifically, for malignant disease, it is well established that TGF-β acts as a tumor suppressor during the initial stages of tumor development, whereas at advanced stages, TGF-β promotes tumor growth and metastatic spread. Thus, it will prove important to consider different aspects of the biology of TGF-β in the development and use of inhibitors of TGF-β actions for the treatment of cancer because blockade of signaling may lead to divergent effects depending on the stage of the disease.

**References**


4. Wana J, Lui, Wieser R, Ventura F, Massagué J. Mechanism of activation of the...


29. Seki T, Hong KH, Oh SP. Nonoverlapping expression patterns of akl and ak5 reveal distinct roles of each receptor in vascular development. Lab Invest. 2006;86(2):116-124.


63. Roman BL, Pham VN, Lawson ND, et al. Disrup- tion of aklr increases endothelial cell number in

60. Hong KH, Seki T, Oh SP. Activin receptor-like kinase 1 is essential for placental vascular development in mice. Lab Invest. 2007;87(7):670-679.


ALK1 as an emerging target for antiangiogenic therapy of cancer

Sara I. Cunha and Kristian Pietras