Clinical Relevance and Mechanisms of Antagonism Between the BMP and Activin/TGF-β Signaling Pathways

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The transforming growth factor β (TGF-β) superfamily is a large group of signaling molecules that participate in embryogenesis, organogenesis, and tissue homeostasis. These molecules are present in all animal genomes. Dysfunction in the regulation or activity of this superfamily's components underlies numerous human diseases and developmental defects. There are 2 distinct arms downstream of the TGF-β superfamily ligands—the bone morphogenetic protein (BMP) and activin/TGF-β signaling pathways—and these 2 responses can oppose one another's effects, most notably in disease states. However, studies have commonly focused on a single arm of the TGF-β superfamily, and the antagonism between these pathways is unknown in most physiologic and pathologic contexts. In this review, the authors summarize the clinically relevant scenarios in which the BMP and activin/TGF-β pathways reportedly oppose one another and identify several molecular mechanisms proposed to mediate this interaction. Particular attention is paid to experimental findings that may be informative to human pathology to highlight potential therapeutic approaches for future investigation.

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The transforming growth factor β (TGF-β) superfamily is a large group of signaling molecules that includes TGF-βs, activins, growth differentiation factors (GDFs), and bone morphogenetic proteins (BMPs).[1] Components of the TGF-β superfamily are present in all animal genomes studied to date, but they are not found outside this kingdom, indicating that it is an ancient intercellular communication pathway in animals. As such, studies in humans and animal models such as nematodes, flies, fish, and rodents, have unequivocally demonstrated that members of this superfamily play conserved roles in embryo specification, organogenesis, and tissue homeostasis. Moreover, dysfunction in the regulation or activity of this superfamily's components underlies numerous human diseases and developmental defects.[2] Cardiovascular, connective tissue, and musculoskeletal diseases seem to be the most common outcomes of altered TGF-β superfamily signaling and include entities such as Marfan syndrome, Camurati-Engleman disease, and fibrodysplasia ossificans progressiva.[2] Vascular diseases, such as hereditary hemorrhagic telangiectasia and pulmonary arterial hypertension, and some hereditary cancers are also related to defects in TGF-β superfamily signaling.[2]

In this review, we identified studies with potential relevance to this topic in PubMed using combinations of the following search terms: *activin*, *BMP*, *bone morphogenetic protein*, *GDF*, *growth differentiation factor*, *TGF*, and *transforming growth factor*. We then selected studies for further examination on the basis of their clinical relevance and experimental demonstration of antagonism between the BMP and activin/TGF-β signaling pathways.

TGF-β Superfamily Signaling Pathway

In humans, the TGF-β superfamily consists of more than 30 secreted ligands that signal through heteromeric combinations of receptor serine/threonine kinases embedded in the cell membrane.[1] A generalized schematic of the TGF-β superfamily signaling is shown in the *Figure*. These receptors are classified into types 1 and 2, of which there are 7 and 5 isoforms, respectively. In the classic pathway, ligand binding brings the constitutively active type 2 receptor near the type 1 receptor, allowing transphosphorylation to occur. The activated type 1 receptor then phosphorylates the C-terminus of a set of effector proteins called receptor-activated SMADs (RA-SMADs), of which there are 5 isoforms, and renders them active. The RA-SMAD then complexes with the transcription factor SMAD4 and translocates to the nucleus to accomplish gene regulation. In addition, several clinically relevant signaling pathways are also activated by TGF-β superfamily ligands, including p38, extracellular signal-regulated kinase, and protein kinase B. SMAD4-independent abilities have also been reported, such as the regulation of microRNA processing.[3]

Given the widespread utility of TGF-β superfamily signaling in humans and other animals, it is not surprising that the activity of this pathway is regulated at many levels.[4] For instance, extracellular antagonists, such as follistatin and noggin, function to sequester ligands upstream of receptor binding and prevent pathway activation. Receptor use by TGF-β superfamily ligands can be modulated by co-receptors such as endoglin. The inhibitory SMADs—SMAD6 and SMAD7—prevent RA-SMAD interaction with SMAD4 or block their activation at the type 1 receptor level, respectively. Receptor-activated SMAD and receptor degradation is promoted by E3 ubiquitin ligases such as SMAD ubiquitination regulatory factor 1 (SMURF1).[1] The transcriptional regulation ability of SMADs can be blocked by interaction with inhibitory proteins such as ski-related novel protein N (SnoN).

Antagonism Between BMP and Activin/TGF-β Signaling

Genomic and genetic studies have revealed that the same basic pathway architecture found in humans is conserved among all other animals. Two distinct signaling pathways are located downstream of the TGF-β superfamily ligands (*Table*). In general, structural considerations delineate the TGF-β superfamily into ligands that interact with ALK1/2/3/6 or ALK4/5/7, the former being mostly BMPs (some of which are also called GDFs) and the latter being mostly activins and TGF-βs. Evolutionarily, it is likely that the BMP response (activation of RA-SMADs 1, 5, and 8) is ancestral, whereas the activin/TGF-β response (activation of RA-SMADs 2 and 3) arose later.[1] However, both pathways converge at the common transcription factor SMAD4, which helps explain the synergistic effects that are often observed upon combined activation. On the other hand, reports of markedly opposing effects between the BMP and activin/TGF-β pathways are becoming increasingly common, especially in disease states, with more than 50 articles published in the past 5 years alone. Most of the studies, though, focus on a single arm of the TGF-β superfamily, leaving the contribution of antagonism unknown in most developmental and physiologic contexts.

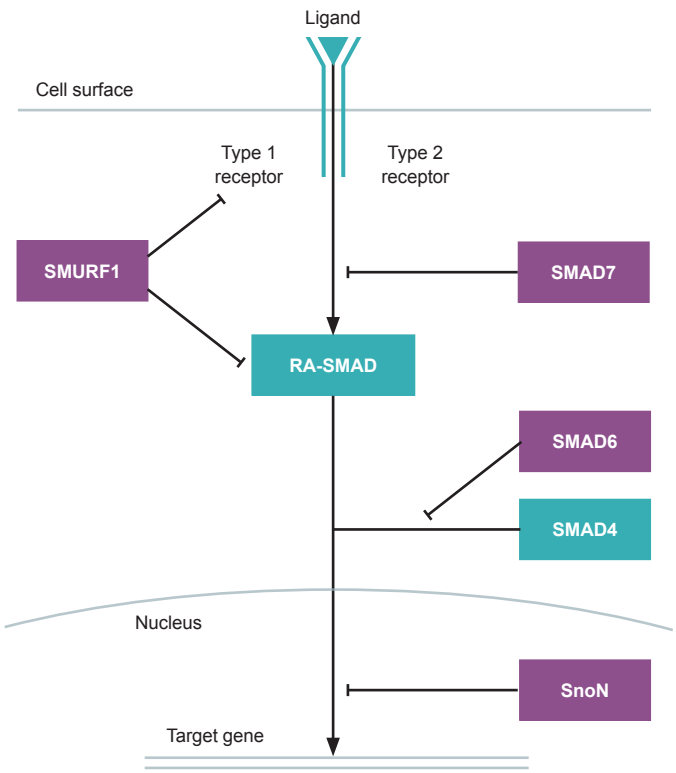

Figure.
Generalized schematic of transforming growth factor β (TGF-β) superfamily signal transduction. Ligands interact with combinations of type 1 and type 2 receptors, which in turn activate a set of effectors called receptor-activated SMADs (RA-SMADs). Receptor-activated SMADs recruit the transcriptional co-factor SMAD4 and translocate to the nucleus to accomplish gene regulation. Receptor-activated SMAD activation is regulated by SMAD7. Recruitment of SMAD4 is regulated by SMAD6. Gene regulation events can be inhibited by ski-related novel protein N (SnoN). Degradation of receptors and RA-SMADs is promoted by SMAD ubiquitination regulatory factor 1 (SMURF1).

Clinical and Physiologic Contexts of Antagonism

Musculoskeletal System

Osteoporosis affects 10 million people in the United States and accounts for 1.5 million fractures annually.[5,6] With an additional 34 million people in the United States at risk for the disease, osteoporosis is both a major health problem and a considerable socioeconomic burden.[5] Osteoporosis-related bone loss occurs when bone resorption exceeds bone formation during remodeling, and a growing body of evidence indicates that activin/TGF-β signaling negatively influences postnatal bone mass. For instance, in vivo studies have shown that when activin/TGF-β signaling is reduced through genetic or pharmacologic approaches, such as neutralizing antibody or receptor decoy strategies, bone formation rate and bone mass increase.[7-13] In contrast, other in vivo studies have demonstrated that BMP signaling in osteoblasts regulates bone formation and that an adequate level of BMP signaling is part of the mechanism for maintaining adult bone mineral density.[14-16] These findings are congruent with the first function attributed to BMP signaling, which is the ability to promote endochondral ossification by inducing chondrocyte and osteoblast differentiation.[17] Conversely, activin/TGF-β signaling can inhibit mineralization of the extracellular matrix, alkaline phosphatase activity, and proteoglycan synthesis, especially at later stages of chondrocyte and osteoblast differentiation.[18-31]

Reciprocal effects of the BMP and activin/TGF-β pathways have also been demonstrated to play a role in regulating skeletal muscle mass and function, which is clinically relevant for patients with sarcopenia.[32] As many as 13% of men and women older than 60 years have this condition, characterized by low skeletal muscle mass and strength.[32] For many years, it has been known that activin and activinlike ligands such as myostatin negatively regulate skeletal muscle mass[33,34]; however, studies published in 2013 demonstrated that BMP signaling positively regulates skeletal muscle mass and is essential to maintain muscle mass after disruption of the

neuromuscular junction.[35,36] These findings are particularly intriguing given that some patients present with osteosarcopenia, in which decreases are seen in both the bone and skeletal muscle mass, and therapeutic strategies targeting the ratio of BMP to activin/TGF-β signaling, such as the receptor decoys or neutralizing antibodies mentioned above, may be beneficial for the simultaneous management of both conditions.

Pulmonary Vasculature

Activin/TGF-β signaling is reportedly elevated in the lungs of patients with pulmonary hypertension.[37] Pulmonary hypertension is a group of diseases characterized by remodeling of the small pulmonary arteries, which leads to increased pulmonary vascular resistance and eventual right ventricular hypertrophy and heart failure. Effective strategies for treating the vascular remodeling in pulmonary hypertension are lacking, in part due to an incomplete understanding of the underlying mechanisms mediating this process.[38]

Numerous lines of evidence from animal studies implicate activin/TGF-β signaling in promoting pulmonary hypertension development.[39-41] Conversely, genetic studies from humans and animals demonstrate that BMP signaling plays an endogenous protective role in maintaining proper pulmonary vascular integrity and endothelial function, and loss-of-function mutations in BMP components have been shown to increase the symptoms and physiologic changes associated with pulmonary hypertension.[4] The reasons for this reciprocal relationship are not clear at present, but detailed studies using genetically modified pulmonary artery vascular smooth muscle cells indicate that BMP signaling opposes the activin/TGF-β pathway–induced effects on proliferation and expression of proinflammatory cytokines such as IL-1 and IL-6.[42] These findings raise the possibility that strategies to activate BMP signaling may be beneficial in ameliorating the activin/TGF-β pathway–induced vascular remodeling in pulmonary hypertension; proof-of-concept for this idea has been provided in a 2015 study in mice.[43]

Tissue Fibrosis

Transforming growth factor β is well recognized to promote fibrosis via epithelial-to-mesenchymal transition in numerous disorders. These disorders include cardiac fibrosis, renal fibrosis, pancreatic fibrosis after repeated episodes of acute pancreatitis, keloid formation, persistent skin fibrosis, chronic liver fibrosis, and pulmonary fibrosis.[44,45] A provocative set of studies has highlighted that the BMP pathway opposes TGF-β–induced fibrosis and promotes tissue recovery in several of these clinically relevant contexts. For example, head-to-head competition between BMP and TGF-β signaling in fibrosis has been reported in cardiomyocytes,[46] ocular burn injuries,[47] silica-induced or allergen-induced pulmonary fibrosis,[48,49] and in a unilateral ureteral obstruction model in which TGF-β promoted glomerular fibrosis.[50] Of note, Manson et al[50] demonstrated that the endogenous BMP pathway plays a critical role in recovery after obstructive uropathies and that the treatment of mice with exogenous BMP7 enhances renal recovery after unilateral ureteral obstruction. These studies serve as substantial proof-of-concept that activation of BMP signaling may be therapeutically advantageous in other contexts of TGF-β–induced fibrosis.

Cellular Differentiation

Antagonism between these pathways also appears to play a role in animal development, with activated SMAD1 (BMP signaling) vs SMAD2 (activin/TGF-β signaling) defining particular zones of endodermal differentiation during anteroposterior patterning in the embryo.[51] Antagonism between BMP and activin/TGF-β signaling has also been reported in fetal trophoblastic and pancreatic epithelial cell differentiation,[52,53] hair follicle stem cell quiescence,[54] fetal pancreatic colony formation,[52] activation of natural killer cells in cancer,[55] and myogenic differentiation of myoblasts.[56]

The ability of antagonism between BMP and activin/TGF-β to affect cellular differentiation may also have

Table 1.
Signal Transduction Components Listed by Predominant Association With BMP or Activin/TGF-β Signaling Pathways

Component	BMP Pathway	Activin/TGF-β Pathway
Representative ligands	BMP2, BMP7	Activin, Myostatin, TGF-β1
Type 1 receptors	ALK1, ALK2, ALK3, ALK6	ALK4, ALK5, ALK7
Type 2 receptors	BMPR2, ACVR2A, ACVR2B	ACVR2A, ACVR2B, TGF-βR2
RA-SMAD	SMAD1, SMAD5, SMAD8	SMAD2, SMAD3
Co-SMAD	SMAD4	SMAD4

Abbreviations: BMP, bone morphogenic protein; RA-SMAD, receptor-activated SMAD; TGF-β, transforming growth factor β.

significant implications for therapeutic strategies involving cellular reprogramming or tissue engineering. For instance, differentiation states of human and mouse embryonic stem cells and human-induced pluripotent stem cells are affected by antagonism between BMP and activin/TGF-β signaling.[57-59]

Antagonistic Mechanisms

Collectively, the studies cited above indicate that antagonism between these pathways occurs in varied cellular and physiologic contexts, many of which are clinically relevant. It should also be noted that the antagonistic relationship operates bidirectionally and in response to numerous ligands. We contend that a better understanding of the molecular mechanisms mediating the interaction between these signaling pathways may identify novel strategies for therapeutic intervention. However, our review reveals that several major gaps exist in the scientific literature and, at present, it is unclear how this widespread antagonism is accomplished at a molecular level.

Perhaps the most straight-forward explanation for how the BMP and activin/TGF-β signaling pathways lead to opposite cellular outcomes would be inversely controlling target gene expression. This mechanism can be thought of like a light switch—with one pathway turning the gene on while the other turns the gene off. This switchlike activity has been demonstrated in several cellular contexts for certain target genes. For instance, TGF-β signaling upregulates the expression of endothelin-1 (*ET-1*), connective tissue growth factor (*CTGF*), plasminogen activator inhibitor-1 (*PAI-1*), e-cadherin, and S100 calcium-binding protein a4 (*S100A4*), but BMP7 downregulates these same targets.[56,60-62] Similar data exist demonstrating the ability of BMP2 to downregulate activin/TGF-β target genes,[23,63] likely indicating a general effect of the BMP pathway rather than BMP ligand specific. The reciprocal relationship has also been demonstrated wherein BMP2-induced genes are downregulated by TGF-β or activin.[23,64] Differential gene regulation likely explains the antagonism between these pathways in certain scenarios; however, we believe it has important limitations. The target genes of the BMP and activin/TGF-β pathways vary widely by physiologic context for reasons that are poorly understood,[65] thus making it difficult to conceive that the reciprocal relationship is consistently maintained. For instance, although BMP and TGF-β oppositely regulate the expression of the inhibitor of differentiation-1 gene (*ID1*), both pathways can also upregulate *ID1* expression[66,67]; said differently, *ID1* expression may be controlled by an "on/on" switch in certain scenarios. Numerous additional examples exist of gene targets that are shared by both pathways while, simultaneously, others are inversely regulated.[23]

Some investigators have postulated that, since SMAD4 is a transcriptional co-factor that is shared between both the BMP and activin/TGF-β signaling pathways, perhaps competition for SMAD4 use underlies the differential responses to these pathways.[35,63] This mechanism may be viewed as a "tug of war" for SMAD4 use, with the dominant pathway winning. However, others have questioned this model by reporting that SMAD4 levels are not limiting[54,68]—there is slack in the rope. And, antagonism between these pathways can be observed at the level of RA-SMAD activation,[35] which is both independent and upstream of SMAD4 recruitment; in other words, the tug of war game can be influenced before anyone gets to the rope in the first place.

We do not favor the idea that differential gene expression or competition for SMAD4 are sufficient explanations on their own for the widespread antagonism between these pathways. An additional possibility is that the BMP and activin/TGF-β pathways interact by decreasing either the strength or persistence of signal transduction in the other pathway. In this model, cellular outcome is a result of the ratio between the 2 pathways, much like a seesaw resting on a fulcrum: one pathway may increase at the expense of the other pathway. Pharmacologic inhibition of the activin/TGF-β pathway has been reported to increase BMP pathway activity (ie, increased levels of activated SMAD1, SMAD5, and SMAD8).[22,69] And, co-treatment with TGF-β reduces the potency of BMP2 or BMP7,[70] whereas loss of the activin/TGF-β effector SMAD3 increases BMP responsiveness.[28] Conversely, loss of the BMP receptor ALK3 potentiates activin/TGF-β signaling.[71] To us, these studies indicate that under certain circumstances, there is tonic repression of activated BMP effector levels by activin/TGF-β signaling, and vice versa. This mechanism would be expected to operate upstream of and influence both of the aforementioned mechanisms. In other words, whichever pathway is heavier on the seesaw could win the tug of war and control the light switch.

This, then, begs the question of how the seesaw is tilted—how is signal transduction in the BMP pathway dampened by activation of the activin/TGF-β pathway and vice versa? One possibility is that one pathway may reduce the expression of components of the other pathway, thereby limiting the amount of signal that is transduced. Several reports found that activin and TGF-β reduce the levels of BMP ligands, receptors, and effectors.[46,70,72,73] This action might be in response to the activity of epigenetic mechanisms such as histone deacetylase activity in conjunction with SnoN or increased protein turnover via SMAD7 function.[24,70,74] The overexpression of SnoN or SMAD7 also impairs the activin/TGF-β pathway, and BMP signaling causes degradation of the TGF-β receptor ALK5 via SMAD7, which raises questions about the specificity of these factors in mediating antagonism between these pathways.[61,62,75,76]

Another possible mechanism is reducing the ability of ligands to engage receptors, which would presumably lead to less signal initiation. For instance, TGF-β and activin each increase the expression of the BMP antagonists gremlin, MGP, and connective tissue growth factor,[72,77,78] each of which sequester BMP ligands and prevent them from interacting with BMP receptors. Transforming growth factor β also induces the expression of the membrane-bound BMP antagonist TMEFF1.[54] In addition, several reports raise the possibility that BMP and activin ligands compete with one another for receptors.[68,79,80] The mechanisms regulating this competition are unclear at present, but a 2015 study by Lowery et al[79] suggests that this competition occurs at a greater degree when the BMP-specific type 2 receptor (BMPR2) is absent, likely because, in the absence of BMPR2, BMP ligands must use the type 2 receptors ACVR2A and ACVR2B, which are the exclusive type 2 receptors for activin ligands.[1] Pretreatment with one class of ligand has been found to decrease the responsiveness to the other, and this effect is abolished when ACVR2 levels are increased.[68,80] This activity suggests that receptor

availability may be limiting in some scenarios, particularly when BMPR2 levels are decreased, such as in pulmonary arterial hypertension.[81] The unique role of BMPR2 is further highlighted by the fact that its presence or absence inversely correlates with TGF-β responsiveness.[42,60,82-85] A notable exception is the postnatal skeleton where, for reasons unknown, loss of BMPR2 expression selectively reduces activin/TGF-β signaling and leads to increased bone mass.[79]

In addition to the evidence detailed above, experimental evidence indicates that the following mechanisms may also exist: the transcription factor GATA4 and the secreted molecule kielin/chordin-like protein enhance BMP signaling while suppressing activin/TGF-β signaling.[86,87] The activin/TGF-β effector SMAD3 forms a nonfunctional transcriptional complex with the BMP effectors SMAD1 and SMAD5 to repress BMP-mediated transcription.[88]

Conclusion

A large and ever-growing body of evidence indicates that proper regulation and function of the BMP and activin/TGF-β signaling pathways is critical for normal human development and health. Much has been learned about the molecular effects of these pathways on tissue homeostasis. In our opinion, an underappreciated theme in the TGF-β superfamily field of study is the fact that the BMP and activin/TGF-β signaling pathways often antagonize one another. We are aware that several mechanisms may be operating simultaneously and may vary by physiologic context. We are hopeful that a better understanding of how these pathways interact in a given scenario could lead to novel treatment strategies for pathologic conditions in which antagonism has been demonstrated, such as age-related osteosarcopenia, fibrosis, and pulmonary hypertension. Additionally, insight with regard to regenerative medicine may be gained that could lead to the use of embryonic or induced pluripotent stem cells for these conditions.

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