Bone Morphogenetic Proteins Signal through the Transforming Growth Factor-β Type III Receptor*§

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The bone morphogenetic protein (BMP) family, the largest subfamily of the structurally conserved transforming growth factor-β (TGF-β) superfamily of growth factors, are multifunctional regulators of development, proliferation, and differentiation. The TGF-β type III receptor (TβRIII or betaglycan) is an abundant cell surface proteoglycan that has been well characterized as a TGF-β and inhibin receptor. Here we demonstrate that TβRIII functions as a BMP cell surface receptor. TβRIII directly and specifically binds to multiple members of the BMP subfamily, including BMP-2, BMP-4, BMP-7, and GDF-5, with similar kinetics and ligand binding domains as previously identified for TGF-β. TβRIII also enhances ligand binding to the BMP type I receptors, whereas short hairpin RNA-mediated silencing of endogenous TβRIII attenuates BMP-mediated Smad1 phosphorylation. Using a biologically relevant model for TβRIII function, we demonstrate that BMP-2 specifically stimulates TβRIII-mediated epithelial to mesenchymal cell transformation. The ability of TβRIII to serve as a cell surface receptor and mediate BMP, inhibin, and TGF-β signaling suggests a broader role for TβRIII in orchestrating TGF-β superfamily signaling.

Members of the transforming growth factor-β (TGF-β)2 superfamily (including the TGF-β, the activin/inhibin, and the bone morphogenetic protein (BMP)/growth differentiation factor (GDF) subfamilies) are involved in many cellular processes including growth regulation, migration, apoptosis, and differentiation (1–4). The BMP subfamily, with 20 members, is the largest and has essential roles in development and well established roles in bone formation (1, 5).

BMP initiates signaling upon ligand binding to the high affinity type I BMP signaling receptors, activin-like receptor kinase-1 (ALK1) (6), ALK2, ALK3, or ALK6 (7). The serine/threonine kinase activity of the type I receptor is activated upon recruitment and phosphorylation by a type II receptor, either the BMP type II receptor (BMPRII), or one of the activin type II receptors (ActRII or ActRIIB) (8). Upon activation the type I receptor phosphorylates the intracellular effector proteins, Smad1/5/8 transcription factors, which complex with the common Smad, Smad4, and enter the nucleus to induce BMP-mediated target gene transcription (1). Whereas most BMPs are able to elicit distinct cellular effects, the mechanism by which a limited number of cell surface receptors mediate these divergent effects remains to be established.

Co-receptors are important components of many signaling pathways (9). The TGF-β type III receptor (TβRIII or betaglycan), endoglin (10), and members of the repulsive guidance molecule family, DRAGON, RGMa, and hemojuvelin (11–13), have been characterized as TGF-β superfamily co-receptors. TβRIII is an abundantly and ubiquitously expressed cell surface receptor that enhances binding of all three isoforms of TGF-β to the TGF-β signaling receptor complex (14), and is required for high affinity cell surface binding of TGF-β2. TβRIII also binds inhibin, another TGF-β superfamily member (15). In addition to directly regulating ligand availability, TβRIII also alters the subcellular localization of the signaling receptor complex through interactions with the PDZ domain containing protein, GIPC (16), and β-arrestin2 (17). The demonstration that TβRIII is required for TGF-β2-stimulated epithelial to mesenchymal transformation (EMT) in vitro (18) and the embryonic lethality of the TβRIII knock-out mouse (19, 20) has fostered consideration of a unique and non-redundant role for TβRIII that is independent of ligand presentation to the kinase receptor complexes.

Several observations suggest that TβRIII may serve as a cell surface receptor for BMP. First, BMP shares structural similarity with ligands known to bind TβRIII (21). Second, TβRIII shares extracellular domain homology with endoglin (22, 23), which binds BMP-2 and BMP-7 in the presence of their respective type II receptors (24). Finally, TβRIII is a heparan sulfate proteoglycan (25, 26) and these glycosaminoglycan modifications have been shown to mediate basic fibroblast growth factor binding to TβRIII (27). As BMP has a strong affinity for heparan sulfate (28), these modifications may confer the ability of TβRIII to bind BMP as well. Here we investigate whether TβRIII functions as a cell surface receptor for BMP.
EXPERIMENTAL PROCEDURES

Cell Culture, Plasmids, Antibodies, and Growth Factors—

COS-7 cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) with 10% fetal bovine serum (Invitrogen). NIH3T3 cells were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum. PC-3 cells were maintained in F12 Kaiighn’s (Invitrogen) supplemented with 10% fetal bovine serum.

Human TβRIIIΔgag was generated using XL- Site directed mutagenesis (Stratagene) to mutate serine 532 to alanine (forward: 5'-CCCTTGGGACACGTCTGGTTGAGACATGCTCCAGA and reverse 5'-CATCTGGAACACCACACTGTCCTACAG), followed by mutating serine 543 to alanine (forward: 5'-GGAGATCCATATCACCCTGGCTCCAGAT and reverse 5'-GCTTTATGAAAGATCTGGAGCCAGGTGATA) to make the double mutant. Plasmids were generous gifts from Kohei Miyazono (ALK3 and ALK6), Petra Knaus (BMPRII), and Fernando Lopez-Casillas (myc-TβRIII extracellular domain deletions) (29).

Adenoviruses for EMT assays were generated using the pAdEasy system (30). All concentrated virions were titered by performing serial dilutions of the concentrated virus and counting the number of GFP-expressing 293 cells after 18–24 h.

Adenoviruses containing sequences for human TβRIII and non-targeting control short hairpin RNA were generated by Dharmaco and inserted into a vector co-expressing the DS-non-targeting control short hairpin RNA (shRNA) or shRNA directed against human TβRIII 24 h after plating. The cells were then incubated for 96 h, serum-starved for 5 h, and treated with the indicated concentrations of rhBMP-2 for 10 min followed by direct lysis. Smad1 phosphorylation was assayed by Western blot with phosho-Smad1 antibody (Cell Signaling), with total Smad1 antibody as a loading control (Cell Signaling).

Virul injections and Collagen Gel Assays—Injections and assays were performed as previously described by Desgrosellier et al. (36) with the exception of the addition of vehicle (bovine serum albumin/HCl), 200 μM TGF-β2, or 5 μM BMP-2, BMP-4, BMP-7, or GDF-5 12 h after placement of the explant on collagen. Each GFP-expressing cell was scored as epithelial, activated, or transformed as described (36). For the total number of explants and cells counted, refer to supplemental Tables 3 and 4.

RESULTS

TβRIII Is a Cell Surface Receptor for BMP-2—To determine whether TβRIII functions as a BMP receptor, we expressed TβRIII, along with the BMP receptors, ALK3, ALK6, or BMPRII, in COS-7 cells, which express low endogenous levels of these cell surface receptors and assessed BMP-2 binding by chemically cross-linking 125I-BMP-2 to binding partners on the cell surface. As expected, 125I-BMP-2 bound to ALK3 and ALK6, but not to BMPRII, which cannot bind ligand on its own (Fig. 1A). 125I-BMP-2 was also detected bound to TβRIII in the presence of ALK3, ALK6, and BMPRII (Fig. 1A, lanes 3, 5, and 7) suggesting that TβRIII binds BMP-2. TβRIII expression also modestly increased BMP-2 binding to ALK3 and ALK6, but did not confer ligand binding to BMPRII (Fig. 1A).

BMPRII binds BMP ligands only when in complex with either ALK3 or ALK6 (8). To determine whether TβRIII affects the ability of BMPRII to bind BMP in the presence of ALK3 or ALK6, we expressed TβRIII with these traditional BMP signaling complexes. As expected, BMPRII could be detected bound to 125I-BMP-2 when co-expressed with ALK3 or ALK6 (Fig. 1B, lanes 1 and 3), and TβRIII did not significantly alter BMP-2 binding to BMPRII (Fig. 1B, lanes 2 and 4).
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To determine whether BMP receptor expression was necessary for the ability of TβRIII to bind BMP-2, we expressed TβRIII alone. In the absence of ALK3 and ALK6, 125I-BMP-2 formed a cross-linked complex with fully processed TβRIII in a dose-dependent fashion (Fig. 1C, left) establishing that BMP-2 is able to bind TβRIII independent of other ligand binding receptors.

BMPs bind heparan sulfate with high affinity (28, 37) and TβRIII is a heparan sulfate and chondroitin sulfate proteoglycan (38). These glycosaminoglycan modifications are important for basic fibroblast growth factor binding to TβRIII (27), but not for TGF-β (38) or inhibin binding (39). To determine whether these glycosaminoglycan modifications were important for BMP binding to TβRIII, we used a mutant of TβRIII in which the serines (Ser-535 and Ser-546) necessary for glycosaminoglycan chain attachment are converted to alanines preventing this modification (TβRIIIΔagag) (38). In these studies, the core protein of TβRIIIΔagag was affinity labeled with 125I-BMP-2 in a dose-dependent fashion (Fig. 1C, right) indicating that the heparan sulfate modifications were not necessary for BMP-2 binding to TβRIII.

TβRIII exists in two forms, a membrane bound form and a soluble form, sTβRIII, derived from ectodomain shedding (38). sTβRIII consists of the extracellular domain of TβRIII and is able to bind TGF-β, sequester ligand from the cell surface receptors, and antagonize TGF-β signaling (38, 40). To determine whether sTβRIII is able to bind BMP-2, we exposed recombinant, purified sTβRIII to 125I-BMP-2. As with membrane-bound TβRIII, sTβRIII was affinity labeled with 125I-BMP-2 in a dose-dependent fashion (Fig. 1D, lanes 2–4), with a BMP-2 binding pattern similar to that of the well characterized TβRIII ligand, TGF-β1 (Fig. 1D, lane 1). These data demonstrate that sTβRIII is able to bind BMP-2 and confirm that the binding of BMP-2 to the extracellular domain of TβRIII is direct.

Kinetics and Affinity of BMP Binding to TβRIII—To characterize the interaction between TβRIII and BMP-2 we used surface plasmon resonance (also known as BIAcore), a sensitive method to measure protein-protein interactions (41, 42). BIAcore has been used to define the interactions of multiple TGF-β superfamily ligands with their receptors (6, 43–45). Both BMP-2 and TGF-β1 were immobilized to a dextran sensor chip and purified sTβRIII was the analyte. Upon mathematically fitting the response curves, the model that best fit the binding of TβRIII to BMP-2 was the bivalent analyte (or avidity) model (Fig. 2 and supplemental Table S1). This model also provided the best fit for TGF-β binding to TβRIII, based on previous BIAcore studies (44) and confirmed here (supplemental Fig. 1). The fit to the bivalent analyte model suggested two ligand binding sites on TβRIII for...
In our studies, we investigated the specificity of BMP interactions with TβRIII, which typically act in the context of ligand presenting core proteins, the best known example of which is endoglin (1). To define the specificity of the interaction of BMP with TβRIII, we performed competition experiments with iodinated 125I-BMP-2 (Fig. 1A), 125I-BMP-4, 125I-BMP-7, and 125I-GDF-5 each formed a cross-linked complex with both the fully processed endogenous form of TβRIII, including those lacking either Binding Regions 1 or 2 and endoglin. These data establish that both BMP-2 and BMP-7 can bind to endogenous TβRIII, with one in the membrane-distal half (Binding Region 1) and one in the membrane-proximal half (Binding Region 2) (Fig. 4A) (29, 47). In contrast to TGF-β, inhibin binds selectively to Binding Region 2 (39). To further investigate BMP binding to TβRIII, we defined the regions of TβRIII that mediate BMP binding. We expressed extracellular domain deletions of TβRIII, including those lacking either Binding Regions 1 or 2 and then assessed their ability to bind BMP-2 and BMP-7. Both 125I-BMP-2 (Fig. 4B) and 125I-BMP-7 (Fig. 4C) exhibited a binding pattern identical to that of 125I-TGF-β1 (Fig. 4D). BMP-2 and BMP-7 bound TβRIII mutants with either Binding Region 2 (Fig. 4, B and C, lanes 4 and 5) or Binding Region 1 (Fig. 4, B and C, lane 6) deleted. In contrast, when portions of both of these regions are deleted, no binding occurred for either BMP-2 or BMP-7 (Fig. 4, B and C, lane 3), providing further support for specific binding of both BMP-2 and BMP-7 to the other TβRIII constructs. In addition, like TGF-β1, BMP-2 and BMP-7 appear to preferentially bind Binding Region 1 (Fig. 4, B and C, compare lanes 5 and 6), which is the region most similar to endoglin. These data establish that both BMP-2 and BMP-7 can bind to either of the two ligand binding motifs of TβRIII, similar to TGF-β1, validating the bivalent model for BMP binding to TβRIII.

**TβRIII Enhances Ligand Binding to ALK-3 and ALK-6**—As a co-receptor, TβRIII has an established role in presenting ligand, leading to enhanced TGF-β binding to TβRII and increasing TGF-β signaling (35), while also enhancing inhibin binding to ActRII to facilitate inhibin-mediated antagonism of activin signaling (15). We observed a slight increase in BMP-2 binding to ALK3 and ALK6 in the presence of TβRIII (Fig. 1A). To determine whether TβRIII alters BMP binding to the BMP signaling receptors, the effect of increasing TβRIII expression
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**A**  
![Diagram](image)  

**B**  
![Diagram](image)  

**C**  
![Diagram](image)  

**D**  
![Diagram](image)  

**E**  
![Diagram](image)  

**FIGURE 3.** Multiple members of the BMP family specifically bind to the core protein of TβRIII.  
**A**, evolutionary tree diagram generated by the MacVector program from NCBI sequence alignment of known TβRIII ligands and the BMP members used in this study. **B**, COS-7 cells expressing wild type (wt) TβRIII or TβRIIIΔagg were exposed to 125I-BMP-2, 125I-BMP-4, 125I-BMP-7, 125I-GDF-5, or 125I-TGF-β1 as indicated and chemically cross-linked followed by immunoprecipitation with an antibody to the cytoplasmic tail of TβRIII. Total cellular TβRIII is shown as an expression control (bottom panel).  
**C** and **D**, COS-7 cells expressing wild type TβRIII (C) or TβRIIIΔagg (D) were simultaneously exposed to 2 nm 125I-BMP-7 in the presence of increasing amounts of cold BMP-7 (0.2, 2, 20, and 200 nm) as indicated, followed by chemical cross-linking and immunoprecipitation.  
**E**, NIH3T3 cells were exposed to 125I-BMP-2, 125I-BMP-7, and 125I-TGF-β1. All lysates were immunoprecipitated with either preimmune serum or a TβRIII antibody, separated by SDS-PAGE, and detected by phosphorimaging. The data are representative of three independent experiments.

don the binding of either 125I-BMP-2 or 125I-BMP-7 to ALK3 and ALK6 was examined. TβRIII significantly increased binding of 125I-BMP-2 to ALK3 in an expression-dependent manner (Fig. 5A), with a maximal 2-fold increase (Fig. 5C). Expression of TβRIII also significantly enhanced 125I-BMP-2 binding to ALK6 ~2-fold (Fig. 5B) and 125I-BMP-7 (Fig. 5D) binding to ALK6 about 3-fold (Fig. 5E). These increases in binding were due to increasing TβRIII expression and not due to altered ALK3 or ALK6 levels. TβRIII expression did not enhance BMP-4 binding to ALK6 (supplemental Fig. 3) nor BMP-2 binding to BMPRII in the absence of either ALK3 or ALK6 (Fig. 1A, lanes 6 and 7), suggesting that TβRIII does not function to confer BMP ligand binding to BMP receptors unable to bind BMP subfamily members independently. Taken together, these data suggest that one function for TβRIII in binding BMP subfamily members is to enhance BMP binding to their respective ligand binding receptors, without altering ligand binding specificity.

**Loss of Endogenous TβRIII Expression Reduces BMP Responsiveness**—TβRIII-mediated ligand presentation to the signaling receptors increases signaling by the respective ligand, as has been demonstrated for TGF-β (35) and inhibin (15). To assess whether TβRIII-mediated BMP presentation to BMP receptors regulated BMP signaling, we used shRNA to decrease endogenous TβRIII expression and assessed effects on BMP-mediated Smad1 phosphorylation. As a model system we used the human prostate cancer cell line, PC-3, which express moderate levels of endogenous TβRIII (data not shown), express BMPRII, ALK3, and ALK6, and are BMP responsive, including BMP-induced Smad1 phosphorylation (48). PC-3 cells were infected with non-targeting control shRNA and human TβRIII shRNA, which concurrently expresses DS-Red fluorophore. Fluorescent images demonstrated similar infection efficiency and phase-contrast images demonstrated similar viability (data not shown). shRNA to TβRIII consistently decreased endogenous

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placed onto collagen gels. Endothelial cells from this AV cushion form a compact epithelial sheet composed of rounded, tightly packed cells on the surface of the collagen, whereas transformed cells are identified morphologically and functionally as those that elongate and invade into the collagen matrix. Endothelial cells in these AV cushions express TβR III, as well as TβR II and TβRI, along with the BMP receptors, BMPRII and ALK3, and undergo EMT in response to exogenous TGF-β (18, 50). In contrast to AV cushion endothelial cells, ventricular endothelial cells lack TβR III and do not undergo EMT, even in response to an excess of TGF-β. However, expression of TβR III in these ventricular endothelial cells results in TGF-β2-induced EMT, demonstrating a unique requirement for TβR III and suggesting a non-canonical role for TβR III in addition to ligand presentation (18).

This in vitro assay system, where EMT is dependent on the presence of TβR III, is currently the only described assay for TβR III signaling (18). To determine whether BMP-2 is also involved in TβR III-mediated transformation, chick ventricular endothelial cells expressing either GFP or TβR III and GFP were incubated with TGF-β2 or BMP-2. Neither ligand alone induced transformation of control infected cells (Fig. 7E). However, expression of TβR III conferred BMP-2- and TGF-β2-induced EMT (Fig. 7E) as measured by a 2-fold increase in the percentage of transformed cells (cells elongating and invading the collagen gel (Fig. 7, C and D)), and a concomitant decrease in the percentage of epithelial cells (cells rounded and remaining on the surface of the gel (Fig. 7, A and B)). Importantly, BMP-2 (5 nM) induced transformation to a similar extent as 200 pm TGF-β2, and both BMP-2- and TGF-β2-induced transformation were entirely dependent on TβR III expression. These data demonstrate that BMP-2 requires TβR III to mediate this biological response, consistent with TβR III and BMP-2 functioning as a receptor-ligand pair. Because other BMP family members also bind to TβR III, we investigated the ability of these BMP members (BMP-4, BMP-7, and GDF-5) to induce EMT in this model system. Surprisingly, only BMP-2 induced EMT, suggesting a specific functional role for BMP-2 binding to TβR III in mediating EMT during heart development (Fig. 7F).
**DISCUSSION**

Here we demonstrate, for the first time, that TβRIII is able to bind BMP, another class of ligands within the TGF-β superfamily. Importantly, TβRIII is able to bind a broad range of BMP ligands, including BMP-2, BMP-4, BMP-7, and GDF-5. We demonstrate that this binding is specific, through both competition studies and studies demonstrating that disruption of both ligand binding domains prevents BMP binding to TβRIII. Functionally, TβRIII serves as a BMP co-receptor by increasing BMP binding to the BMP signaling receptors, ALK3 and ALK6, and increasing BMP signaling, and as a BMP receptor in a biologically relevant system. Whereas glycosylphosphatidylinositol-linked co-receptors have recently been identified for BMP (DRAGON, RGMa, and hemojuvelin) these receptors each have limited tissue distribution (12). In contrast, TβRIII is a ubiquitously expressed transmembrane receptor (51). TβRIII has previously been identified as a co-receptor for both TGF-β and inhibin based on its ability to bind these ligands and enhance their cellular effects. Data presented here suggest that TβRIII functions similarly for the BMP family of ligands. Given the broad tissue distribution of TβRIII and its ability to bind all major classes of ligands in the TGF-β superfamily, TβRIII is poised to act as a major orchestrator of TGF-β superfamily signaling. As members of the TGF-β superfamily are able to antagonize each other, including BMP-7 antagonism of TGF-β-induced fibrogenesis in mesengial cells (52) and distinct inhibin antagonism of activin and BMP signaling (53), TβRIII may be the common component for these TGF-β superfamily ligands mediating this antagonism. Consistent with this hypothesis, we have demonstrated that BMP-2 can compete with TGF-β1 for binding to TβRIII (data not shown). Current studies are delineating the hierarchy of ligand binding to TβRIII to further define the role of TβRIII as a moderator of TGF-β superfamily signaling.

Here we demonstrate that BMP-2 binding to TβRIII elicits a functional response in ventricular endothelium. These data are consistent with a suggested role for BMP-2 in normal cardiac development. A recent report demonstrated that conditional ablation of BMP-2 in heart muscle, where it is expressed during development, results in failure of the TβRIII expressing endothelial cells in the adjacent valve forming region of the heart to undergo EMT (54, 55). Furthermore, we show that TβRIII has enhanced functional interaction with ALK3 in the presence of BMP-2. Conditional ablation of ALK3 from the endothelium also results in a failure of EMT in the valve forming regions (50). Taken together, these data suggest that BMP-2 produced by the myocardium may act directly through TβRIII on endothelial cells in the heart to stimulate EMT and valvulogenesis.
The differences noted between BMP ligand binding to TβRIII and the biological effect of these BMP ligands may be explained by the relative expression level of the ligands and the identity of the receptors recruited into the signaling complex. The related TGF-β superfamily co-receptor, endoglin, participates in differentially activating ALK1 and ALK5 in endothelial cells, with low TGF-β concentrations activating ALK1 and stimulating angiogenesis, whereas higher concentrations activate ALK5 to inhibit angiogenesis (56–58). The present data demonstrate that BMP-2 can cause functional recruitment of TβRIII to ALK3 consistent with a role for ALK3 in stimulating endothelial cell transformation. In contrast, TβRIII leads to enhanced BMP-7 binding to ALK6, which is not expressed in the endothelial cell model used here (50, 59). Thus, these differences in receptor expression may account for the differences in functional response between BMP-2 and the other ligands (BMP-4, BMP-7, and GDF-5) and supports a model where specific ligands may initiate the assembly of particular receptor complexes to generate diversity in biological effect.

Members of the BMP family activate cellular responses through the formation of an active signaling receptor complex containing the type I and type II receptors (8). BMP binding data presented here suggest that TβRIII does not alter the formation of this active complex. Future studies will be aimed at characterizing the effect of TβRIII on the formation, stability, and activity of the signaling complex and determine whether TβRIII alters ligand binding to the other BMP type II receptors, including ActRII and ActRIIB.

A number of BMP family members, including BMP-2, BMP-4, and BMP-7, have been associated with heart development. Here we demonstrate that TβRIII is uniquely essential for BMP-2-induced EMT in the developing heart, as TβRIII expression, did not confer BMP-4- or BMP-7-induced EMT. Our data are consistent with recently published data indicating that BMP-4 is dispensable for EMT in the heart (60) and that BMP-7 antagonizes TGF-β1-induced EMT, whereas having no effect on EMT by itself (61). Thus, EMT is not a physiologically relevant assay for BMP-4 and BMP-7. These data further support the hypothesis that TβRIII does not confer BMP function, but facilitates their function. Further investigation will be required to define the effect of TβRIII on other BMP family ligands in physiologically relevant assays.

Binding of BMP to TβRIII occurs through two ligand binding domains on the core protein of TβRIII, similar to TGF-β, and...
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does not require the heparan sulfate and chondroitin sulfate modifications to the extracellular domain of TβRIII. Whereas our data indicate that the glycosaminoglycan chains are not necessary for BMP binding, they may still contribute to enhance or alter ligand binding. The mechanisms regulating post-translational processing of TβRIII are not well understood and likely to be cell type-specific. The effect of differential post-translational processing on altering interactions of TGF-β superfamily members with TβRIII warrants additional investigation.

In addition to glycosaminoglycan modifications, the extracellular domain of TβRIII is proteolytically cleaved from the membrane and shed into the extracellular environment. Here we demonstrate that recombinant, purified sTβRIII can bind BMP, indicating that the interaction is direct and that anchorage to the cell membrane and proximity of other BMP binding components are not necessary for ligand binding. BMP is secreted into the extracellular environment as an active ligand and the bioavailability of BMP is tightly controlled by a number of soluble BMP antagonists that bind ligand and sequester BMP from the signaling receptors, including Noggin, Chordin, Floodstatin, and gremlin (4, 7). The ability of sTβRIII to bind BMP-2 suggests that sTβRIII may be an additional mechanism by which the bioavailability of BMPs is regulated.

Expression of both BMP and TβRIII are essential during embryonic development. Here we demonstrate that TβRIII is important for BMP-induced EMT in ventricular cells of the chick heart. Therefore, defects in development of the TβRIII knock-out mice may not only be due to alterations in TGF-β signaling, but also alterations in BMP signaling. In addition to the role of BMP in development, alterations in the BMP signaling pathway have been linked to a number of hereditary human diseases, including primary pulmonary hypertension, juvenile polyposis syndrome, ovarian dysgenesis 2, and A2 brachydactyly (62, 63). BMP signaling also has an emerging role in regulating cancer biology with effects on alterations in BMP signaling. In addition to the role of BMP in cancer development. Here we demonstrate that loss of TβRIII expression results in a decrease in cellular sensitivity to BMP, as assayed by Smad1 phosphorylation. Further defining the contribution of TβRIII to BMP signaling will aid in establishing the mechanism by which TβRIII functions during tumorigenesis, and whether alterations in TβRIII expression or function are linked to other human diseases.

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