Transforming Growth Factor (TGF-β)-specific Signaling by Chimeric TGF-β Type II Receptor with Intracellular Domain of Activin Type IIB Receptor*

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Members of the transforming growth factor-β (TGF-β) superfamily signal via different heteromorphic complexes of two sequentially acting serine/threonine kinase receptors, i.e. type I and type II receptors. We generated two different chimeric TGF-β superfamily receptors, i.e. TfβR-I/BMPR-IB, containing the extracellular domain of TGF-β type I receptor (TβR-I) and the intracellular domain of bone morphogenetic protein type IB receptor (BMPR-IB), and TfβR-II/ActR-IIB, containing the extracellular domain of TGF-β type II receptor (TβR-II) and the intracellular domain of activin type IIB receptor (ActR-IIIB). In the presence of TGF-β1, TβR-I/BMPR-IB and TβR-II/ActR-IIB formed heteromeric complexes with wild-type TβR-II and TβR-I, respectively, upon stable transfection in mink lung epithelial cell lines. We show that TβR-II/ActR-IIB restored the responsiveness upon transfection in mutant cell lines lacking functional TβR-II with respect to TGF-β-mediated activation of a transcriptional signal, extracellular matrix formation, growth inhibition, and Smad phosphorylation. Moreover, TβR-I/BMPR-IB and TβR-II/ActR-IIB formed a functional complex in response to TGF-β and induced phosphorylation of Smad1. However, complex formation is not enough for signal propagation, which is shown by the inability of TβR-I/BMPR-IB to restore responsiveness to TGF-β in cell lines deficient in functional TβR-I. The fact that the TGF-β1-induced complex between TβR-II/ActR-IIB and TβR-I stimulated endogenous Smad2 phosphorylation, a TGF-β-like response, is in agreement with the current model for receptor activation in which the type I receptor determines signal specificity.

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§ The abbreviations used are: TGF-β, transforming growth factor-β; TβR, TGF-β receptor; ActR, activin receptor; BMP, bone morphogenetic protein; BMPR, BMP receptor; GS domain, glycin-serine-rich domain; FBS, fetal bovine serum; OP, osteogenic protein; PAGE, polyacrylamide gel electrophoresis; PAI, plasminogen activator inhibitor; PCK, polymerase chain reaction; Smad, Sm and MAD-related protein; DMEM, Dulbecco’s modified Eagle’s medium.
recognize TgR-I as a substrate (28). The notion that type I receptors act downstream of type II receptors is supported by the ability of type I receptors to determine distinct signaling responses (29, 30) and by constitutively active mutants of TgR-I and ActR-IIB to signal in the absence of ligand and type II receptor (22, 24, 25).

Members of the Smad family of proteins have been shown to play a key role in the intracellular signaling pathways of TGF-β superfamily members. After activation by serine/threonine kinase receptors, Smads proteins become phosphorylated and translocate to the nucleus, where they may play a role in the transcriptional regulation (31–35). Smad1 and Smad5 are phosphorylated and translocated into the nucleus upon BMP receptor activation (34, 35), whereas TGF-β and activin induce the phosphorylation and translocation of both Smad2 and Smad3 (36–38).

Previously, we and others have used chimeras of type I and type II receptors to demonstrate that the intracellular domains of type I and type II receptors each serve distinct roles in signaling (39–43). In the present study, we investigated the functional properties of chimeric receptors in which the intracellular domains of type I or type II receptors for TGF-β were replaced with the corresponding domains of BMPR-IB and ActR-IIB, respectively. Our data indicate that the intracellular domain of TgR-II can be replaced by the intracellular domain of ActR-IIB, which is a receptor for activin as well as for BMP (44), with retained ability to activate TgR-I and to induce TGF-β-like responses. In contrast, TgR-I/BMPR-IB was unable to induce any signal in complex with TgR-I, although it was shown to be functional in complex with TgR-II/ActR-IIB in both a transcriptional activation assay and in a Smad phosphorylation assay. This indicates that complex formation is necessary but not sufficient for signal transduction. Moreover, the ligand-induced phosphorylation of endogenous Smad2, but not of endogenous Smad5, by TgR-II/ActR-IIB in complex with TgR-I confirmed the notion that the signal specificity is controlled by the type I receptor.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—COS-1 cells and Mv1Lu cells were obtained from American Type Culture Collection. Mv1Lu cells that lack functionally active TgR-I (4–2 mutant cells) or TgR-II (DR 26 mutant cells; Dr. J. Massague) (8) were provided by Dr. J. Massague. Cells were cultured in 5% CO2 at 37 °C in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Inc.) with 10% fetal bovine serum (FBS); Life Technologies, Inc.), 100 units of penicillin, and 50 μg/mL streptomycin.

**Construction of Chimeric and Wild-Type Receptors**—We employed a two-step polymerase chain reaction (PCR) using a Perkin-Elmer thermal cycler with Pyrococcus furiosus DNA polymerase (Stratagene) to generate the chimeric receptors (Fig. 1). cDNAs for human TgR-I (10), mouse BMPR-IB (12), human TgR-II (13), and mouse ActR-IIB (15) were used as templates. For the TgR-I/BMPR-IB chimera, in the first PCR step primer pairs 21 (sense; 5′-GGGAAATTCCGCGCTGCATGGCGG-3′) and 22 (antisense; 5′-GAGGGTCATCCGAGCTGAGG-3′) were used with TgR-II as template, and primer pairs 23 (sense; 5′-CTACTGCGATCCGACAACTG-3′) and 24 (antisense; 5′-CCGGAGAAGGAGCTTCCAGG-3′) were used with ActR-IIB as template. Primers 22 and 23 are complementary to each other (underlined sequences), and primer 21 contains an extra 5′-EcoRI restriction site. In the second PCR step, the two PCR products were used as template along with the terminal primers 21 and 24; the chimeric type II receptor PCR product was subcloned, and the DNA was sequenced. Subsequently, the EcoRI-SacI PCR fragment of TgR-II/ActR-IIB encoding the N-terminal part was ligated together with the C-terminal part of ActR-IIB to subsequence subcloning into pME4. Wild-type mouse BMPR-IB, human TgR-II, mouse ActR-IIB, and human TgR-I were subcloned in pME4 using convenient restriction enzyme cutting sites. Receptor expression thereby came under the transcriptional control of the ZnCl2-inducible human metallothionein promoter.

**Transfection**—R 4–2 and DR 26 mutant cells were stably transfected by the calcium phosphate precipitation method using the MBS mamalian transfection kit (Stratagene), following the manufacturer's protocol. Selection of transfectants was performed in the presence of 100 units/ml hygromycin (Sigma). We obtained cell pool cultures with similar levels of receptor expression upon ZnCl2 treatment.

**Binding, Affinity Cross-linking, and Immunoprecipitation—TGF-β1 was iodinated by the chloramine T method (45). Binding and affinity cross-linking using disuccinimidyl carbonate (Pierce) were performed as described (10). Lysates were prepared from affinity cross-linked cells and subjected to immunoprecipitation, using antisera against TgR-I (VPN1, 40), TgR-II (DR1) (46), BMPR-IB (47), hemagglutinin epitope (12CA5, Babco, Ref. 48), or His epitope (HSV) (gift from Dr. T. K. Sampath); samples were analyzed by SDS-PAGE using 4–15% gradient gels and visualized using a Fuji-X Biolmage.

**Growth Inhibition Assay**—Cells were seeded at a density of 1.5 × 10^4 cells/well in 24-well plates in DMEM with 10% FBS. After 24 h, cells were washed once, and medium was changed to DMEM with 3% FBS and 100 μM ZnCl2, and cells were then incubated with different concentrations of TGF-β1 for 22–24 h; during the last 2 h, cells were labeled with 1 μCi/ml [3H]thymidine (Amersham Corp.). Thereafter, the cells were fixed in 5% ice-cold trichloroacetic acid for 20 min, washed with 5% trichloroacetic acid followed by water and 70% ethanol, and finally solubilized in 0.1 n NaOH. 3H radioactivity was measured in a liquid scintillation β-counter using Ecosint (National Diagnostics).

**Extracellular Matrix Formation Assay**—Cells were seeded in six-well plates at a density of 1 × 10^4 cells/well. After 18–24 h, the medium was changed to DMEM supplemented with 0.1% FBS, with or without 100 μM ZnCl2. After 15 h, the medium was changed to methionine-free MCDB medium (SVA, Sweden) with different concentrations of TGF-β1 and incubation prolonged for 6 h; during the last 2 h, cells were incubated with 25 μCi/ml [35S]-labeled Proline (Amersham Corp.). For extracellular matrix isolation, the cells were removed by washing in ice; once in phosphate-buffered saline, three times in 10 mM Tris-HCl, pH 8.0, 0.5% sodium deoxocholate, 1 mM phenylmethylsulfonyl fluoride; twice in 20 mM Tris-HCl, pH 8.0; and once in phosphate-buffered saline. Extracellular matrix proteins were scraped off and extracted into SDS sample buffer containing 10 mM dithiothreitol.

![Fig. 1. Schematic representation of the chimeric receptors used in the present study.](image-url)

**FIG. 1.** Schematic representation of the chimeric receptors used in the present study. Extracellular domains, transmembrane regions, and intracellular domains are indicated. TgR-II and ActR-IIB share 33.3% sequence identity, and TgR-I and BMPR-IB share 59.8% sequence identity in their intracellular domains. αα, amino acids.
Secreted proteins and extracellular matrix proteins were analyzed by SDS-PAGE, followed by fluorography using Amplify (Amersham Corp.) and quantification using a Fuji-X BioImager. PAI-1 was identified as a 45-kDa protein in the extracellular matrix fraction (49).

**Transcriptional Response Assay—** Stable transfectants were transiently transfected with p3TP-Lux (28), as described above. The following day, cells were washed extensively with phosphate-buffered saline to remove calcium phosphate precipitates. Subsequently, the cells were incubated in DMEM with 10% FBS for 16–20 h. Thereafter, the receptor expression was induced by treatment of the cells

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**Fig. 2. Expression and ligand binding to wild-type and chimeric receptors.**

A. R 4–2 mutant cells were stably transfected with wild-type TβR-I (hemagglutinin-tagged), TβR-I/BMPR-IB, and BMPR-IB in pMEP4. Cell pools were 35S-labeled, treated with 100 μM ZnCl2, and immunoprecipitated with anti-TβR-I or anti-BMPR-IB antibodies. Immunoprecipitates were resolved by SDS-PAGE followed by fluorography. The binding of 125I-TGF-β1 to TβR-I and TβR-I/BMPR-IB in stably transfected R 4–2 mutant cells (B) and to TβR-II and TβR-II/ActR-IIIB in stably transfected DR 26 mutant cells (C) and the binding of 125I-BMP-7/OP-1 to ActR-IIIB and BMPR-IB in stably transfected DR 26 mutant cells and R 4–2 mutant cells (D), respectively, were analyzed (without and with ZnCl2 treatment) by affinity cross-linking and subsequent immunoprecipitation using the indicated antisera followed by SDS-PAGE and visualization using a Fuji-X Biolmager. I and II indicate the position of type I and type II receptors, respectively.
Cells were labeled for 18 h in the indicated concentrations. The transcriptional response was determined by measuring the luciferase activity.

RESULTS

Ligand Binding Properties of Chimeric Receptors—Two chimeric receptors were constructed, i.e. T\(b\)R-I/BMPR-IB, containing the extracellular domain of T\(b\)R-I and intracellular domain of BMPR-IB, and T\(b\)R-II/ActR-IIB, containing the extracellular domain of T\(b\)R-II and the intracellular domain of ActR-IIB (Fig. 1). To investigate the \(^{125}\text{I}-\text{TGF-\(b\)}\) binding properties of the chimeric receptors, we used Mv1Lu cells that lack functional T\(b\)R-I (R 4–2 mutant cells) or T\(b\)R-II (DR 26 mutant cells) as host cells for transfection. T\(b\)R-I/BMPR-IB was stably transfected into R 4–2 mutant cells, along with T\(b\)R-I and BMPR-IB as positive and negative controls, respectively. T\(b\)R-II/ActR-IIB was stably transfected in DR 26 mutant cells, along with T\(b\)R-II and ActR-IIB as positive and negative controls, respectively. All receptor expression constructs were placed under the transcriptional control of the metallothionein promoter, which can be induced by ZnCl\(_2\). Expression of type I receptors in stable transfectants was analyzed by metabolic labeling using specific antibodies, directed against the intracellular domains of the receptors. For all transfectants, proteins with expected molecular weights were observed upon the addition of 100 \(\mu\)M ZnCl\(_2\) (Fig. 2A). As observed before, due to the leaky character of the metallothionein promoter, the receptors were also expressed, but at lower levels, in the absence of ZnCl\(_2\) treatment. We observed no co-immunoprecipitation of endogenous type II receptors with anti-type I antibodies, suggesting that there was no ligand-independent type I-type II complex formed as a result of overexpression (Fig. 2A). Expression of type II receptors could not be analyzed by metabolic labeling due to the weak affinity of the antisera.

Affinity cross-linking with \(^{125}\text{I}-\text{TGF-\(b\)}\) of T\(b\)R-I/BMPR-IB cells revealed that T\(b\)R-I/BMPR-IB bound \(^{125}\text{I}-\text{TGF-\(b\)}\) with similar efficiency as T\(b\)R-I. Immunoprecipitation with anti-BMPR-IB antisera not only brought down the T\(b\)R-I/BMPR-IB, but also T\(b\)R-II, illustrating that T\(b\)R-II formed a complex with T\(b\)R-I/BMPR-IB (Fig. 2B). We were not able to detect neither T\(b\)R-II nor T\(b\)R-I/BMPR-IB with T\(b\)R-II antisera (data not shown). As expected, T\(b\)R-I-transfected cells cross-linked T\(b\)R-II complexes could be demonstrated by immunoprecipitation using antisera against T\(b\)R-I or T\(b\)R-II, albeit with less efficiency of complex precipitation with T\(b\)R-II antisera. In nontransfected R 4–2 mutant cells, no type I receptor cross-linked complex was immunoprecipitated with anti-T\(b\)R-I or anti-BMPR-IB antisera (Ref. 10 and data not shown). Although we have reported that BMPR-IB can bind TGF-\(b\)1 when overexpressed in COS-1 cells (12), the expression levels in transfected Mv1Lu cells were too low for this interaction to occur (data not shown).

Affinity cross-linking with \(^{125}\text{I}-\text{TGF-\(b\)}\) of cells transfected with T\(b\)R-II/ActR-IIB or T\(b\)R-II revealed that T\(b\)R-II/ActR-IIB bound \(^{125}\text{I}-\text{TGF-\(b\)}\) equally efficient as T\(b\)R-I upon induction of their expression with ZnCl\(_2\) treatment. T\(b\)R-II/ActR-IIB, like T\(b\)R-I, induced the binding to endogenously expressed T\(b\)R-I (Fig. 2C). In T\(b\)R-II/ActR-IIB cells, cross-linked complexes of T\(b\)R-II/ActR-IIB and T\(b\)R-I were immunoprecipitated with antisera against either ActR-IIB or T\(b\)R-I, indicating that both receptors are part of a common TGF-\(\beta\)-induced receptor complex (Fig. 2C). In the nontransfected DR mutant cells, no type II cross-linked complex could be immunoprecipitated with antisera against T\(b\)R-II or ActR-IIB (data not shown). As expected, no binding of TGF-\(b\)1 to cells stably transfected with
**Figure 4.** Signaling activity of wild-type and chimeric receptors using a PAI-1 assay. R 4–2 mutant cells stably transfected with wild-type TβR-I, wild-type BMPR-IB, or TβR/IBMPR-IB (A) and DR 26 mutant cells stably transfected with wild-type TβR-II, wild-type ActR-IIB, or TβR-II/ActR-IIB (B) were analyzed with respect to induction of PAI-1 protein levels upon TGF-β1 stimulation in the absence or presence of ZnCl₂ treatment. Cells were 35S-labeled with “Promix” for the last 2 h of incubation, and production of 45 kDa PAI-1 protein in extracellular matrix was analyzed by SDS-PAGE followed by fluorography and quantification using a Fuji-X BioImager.

**Table 1.** Signaling activity of wild-type TGF-β receptors using a PAI-1 assay.

| cDNA | TβR-I | TβR-I/IBMPR-IB | BMPR-IB |
|------|-------|----------------|---------|
| TGF-β1 (ng/ml) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |

**Table 2.** Signaling activity of chimeric receptors using a PAI-1 assay.

| cDNA | TβR-IB | TβR-II/ActR-IIB | ActR-IIB |
|------|--------|-----------------|---------|
| TGF-β1 (ng/ml) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |

ActR-IIB was observed (data not shown).

Affinity cross-linking with 125I-BMP-7/OP-1 of cells transfected with BMPR-IB or ActR-IIB revealed that both receptors were expressed and able to bind ligand upon ZnCl₂ treatment (Fig. 2D).

**Signaling Properties of Chimeric Receptors**—The signaling activity of the chimeric receptors was investigated using the p3TP-Lux transcriptional activation assay, which scores positive after stimulation with TGF-β (9) and, albeit less efficiently, after BMP or activin stimulation. Previously, we have shown that ActR-IIs and BMPR-IB can form a BMP-7/OP-1-induced heteromeric complex, which can mediate a p3TP-Lux signal (10). No transcriptional response could be observed after TGF-β1 stimulation when the reporter plasmid was transfected alone into COS-1 cells (Fig. 3A). However, when we co-transfected TβR-I/IBMPR-IB together with TβR-II/ActR-IIB and p3TP-Lux into COS-1 cells, we observed a TGF-β-dependent signal (Fig. 3A). To elucidate the ability of the complex between TβR-I/IBMPR-IB and TβR-II/ActR-IIB to induce Smad phosphorylation, COS-1 cells were transiently co-transfected with TβR-I/IBMPR-IB and TβR-II/ActR-IIB together with Smad1 or Smad2. TGF-β1 induced the phosphorylation of Smad1, but not of Smad2 (Fig. 3B). This indicates that TβR-I/IBMPR-IB and TβR-II/ActR-IIB are functionally intact and can form a TGF-β-mediated complex, which signals a BMP-like response.

We then investigated whether TβR-I/IBMPR-IB and TβR-II/ActR-IIB could substitute for TβR-I and TβR-II, respectively, using TGF-β-induced plasminogen activator inhibitor-1 (PAI-1) production as an assay. Wild-type Mv1Lu cells responded to TGF-β1 by producing a 45-kDa PAI-1 protein, whereas the R 4–2 mutant and DR 26 mutant cell lines did not produce PAI-1 after stimulation by TGF-β1 (data not shown). Sometimes the PAI-1 protein appeared as two discrete bands of 45 and 43 kDa, of which the latter is likely to be a proteolytic breakdown product of the 45-kDa protein. Stably transfected TβR-I/IBMPR-IB R 4–2 mutant cells did not produce the PAI-1 protein upon induction of receptor expression by ZnCl₂ and TGF-β1 stimulation (Fig. 4A). TGF-β1 stimulation of the R 4–2 mutant cells transfected with TβR-I, but not BMPR-IB, led to a 2.5-fold increase, determined by densitometric scanning, in the production of PAI-1 in response to ZnCl₂ treatment, as reported before (10, 12). However, TβR-II/ActR-IIB restored the TGF-β1-mediated PAI-1 response in DR 26 mutant cells to a similar extent as TβR-II, i.e. 5- and 6-fold increases, respectively (Fig. 4B). ActR-IIB, which cannot bind TGF-β1, was unable to complement the defect in DR 26 mutant cells (Fig. 4B). In ActR-IIB-transfected cells treated with ZnCl₂, a weak 45-kDa protein was observed. However, the production of this protein was not induced upon TGF-β1 stimulation.

In addition, we measured the ability of both chimeras to mediate growth-inhibitory responses upon stimulation with TGF-β1. Similar to the p3TP-Lux and PAI-1 assays, TβR-II/ActR-IIB was able to replace TβR-II, but TβR-I/IBMPR-IB was not able to replace TβR-I, with respect to the antiproliferative response upon TGF-β1 stimulation (Fig. 5). In a few experiments, we observed that the degree of growth inhibition was less with TβR-II/ActR-IIB compared with TβR-II.

Different Smads have been shown to act downstream of BMP and TGF-β receptors (34, 37). Thus, we used differential activation of Smad2 or Smad5 to distinguish TGF-β versus BMP receptor-mediated signaling, respectively. Using the stably
transfected cell lines, the effect of ligand-induced complex formation between T\(\beta\)R-II and T\(\beta\)R-I/BMPR-IB, or between T\(\beta\)R-II/ActR-IIB and T\(\beta\)R-I, on endogenous Smad2 or Smad5 phosphorylation was analyzed (Fig. 6). Phosphorylation of Smad2, but not Smad5, was induced by the activated complex between T\(\beta\)R-II/ActR-IIB and T\(\beta\)R-I, whereas ligand-induced complex formation between T\(\beta\)R-I/BMPR-IB and T\(\beta\)R-II induced no Smad2 or Smad5 phosphorylation. BMP-7/OP-1 was found to induce the phosphorylation of Smad5 in Mv1Lu cells; phosphorylated Smad2 and Smad5 were found to co-migrate on SDS-PAGE.² Smad5 antibody also brought down a phosphoprotein larger than Smad5, the identity of which is unknown.

Thus, our results indicate that complex formation between the intracellular domains of ActR-IIB and T\(\beta\)R-I leads to the transduction of TGF-\(\beta\)-like signals, whereas complex formation between the intracellular domains of T\(\beta\)R-II and BMPR-IB does not lead to any measurable signaling event (Fig. 7).

DISCUSSION

TGF-\(\beta\) superfamily members exert their cellular effects through formation of hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. Previous reports have shown that the type II receptor has a constitutively active kinase domain (20), and that phosphorylation and activation of the type I receptor is sufficient and necessary for downstream signaling activities (25, 26). Here we have characterized the properties of two chimeric receptors in which the intracellular domains of different type I or type II receptors within the TGF-\(\beta\) superfamily were exchanged. The purpose was to investigate whether the intracellular domains of TGF-\(\beta\) type I and type II receptors could be replaced by the corresponding domains of BMPR-IB and ActR-IIB, respectively. The presented data show that the intracellular domain of ActR-IIB can functionally substitute for the intracellular domain of T\(\beta\)R-II with respect to the induction of a transcriptional activation signal, stimulation of PAI-1 production, growth inhibition, and Smad2 activation. In contrast, a complex between the intracellular domains of BMPR-IB and T\(\beta\)R-II induced no observable signal (Fig. 7).

T\(\beta\)R-II/ActR-IIB can complement the lack of T\(\beta\)R-II in DR 26 mutant cells. The intracellular domain of ActR-IIB shares 33.3% sequence identity with T\(\beta\)R-II and is thus apparently sufficiently similar to phosphorylate and transactivate T\(\beta\)R-I in a similar way as T\(\beta\)R-II. Previously, we and others have shown that the intracellular domain of T\(\beta\)R-I cannot replace the intracellular domains of T\(\beta\)R-II in this respect (39–42).

Our findings are in agreement with those of Muramatsu et al. (43), who reported the signaling activities of chimeric human...
granulocyte-macrophage colony-stimulating factor receptor/ActR-II and human granulocyte-macrophage colony-stimulating factor receptor/BMPR-II.

The failure of TβR-I/BMPR-IB to signal in combination with TβR-II is unlikely to be due to a perturbation of the conformation of TβR-II extracellular domain or BMPR-IB intracellular domain, since TβR-I/BMPR-IB bound TGF-β and formed a heteromeric complex with TβR-II and since TβR-I/BMPR-IB together with TβR-II/ActR-IIB transduced a TGF-β-dependent transcriptional activation signal and induced Smad1 phosphorylation, a BMP-like signal. It is possible that the inability of TβR-I/BMPR-IB to mediate a growth-inhibitory response may be in an inherent property of the receptor, since Mv1Lu cells, which express TβR-II, TβR-I, ActR-I, and BMPR-IB among other receptors, are at least 100-fold more sensitive to TGF-β than BMP-7/OP-1 with respect to growth inhibition. On the other hand, TGF-β-induced complex formation between TβR-II and TβR-I/BMPR-IB did not lead to Smad2 or Smad5 phosphorylation, suggesting that this is an inactive complex.

Phosphorylation of endogenous Smad2 was stimulated by TGF-β-induced complex formation between TβR-I and TβR-II/ActR-IIB, indicating that type I receptor is the determinant for signal specificity. Our future studies will be aimed at elucidating which minimal specific regions/residues in the intracellular domain of TβR-I need to be substituted for analogous regions/residues in the intracellular domain of BMPR-IB to allow for a TβR-I/BMPR-IB chimera to transduce signals when activated by TβR-II.

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