Function of the Type V Transforming Growth Factor β Receptor in Transforming Growth Factor β-induced Growth Inhibition of Mink Lung Epithelial Cells

Qianjin Liu, Shuan Shian Huang, and Jung San Huang‡

From the Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, Missouri 63104

The type V transforming growth factor β (TGF-β) is a 400-kDa nonproteoglycan membrane protein that co-expresses with the type I, type II, and type III TGF-β receptors in most cell types. The type V TGF-β receptor exhibits a Ser/Thr-specific protein kinase activity with distinct substrate specificity (Liu, Q., Huang, S. S., and Huang, J. (1994) J. Biol. Chem. 269, 9221–9226). In mink lung epithelial cells, the type V TGF-β receptor was found to form heterocomplexes with the type I TGF-β receptor by immunoprecipitation with antiserum to the type V TGF-β receptor after 125I-TGF-β affinity labeling or Trans35S-label metabolic labeling of the cells. The kinase activity of the type V TGF-β receptor was stimulated after treatment of mink lung epithelial cells with TGF-β. TGF-β stimulation resulted in the growth inhibition of wild-type mink lung epithelial cells and to a lesser extent of the type I and type II TGF-β receptor-defective mutants, although higher concentrations of TGF-β were required for the growth inhibition of these mutants. TGF-β was unable to induce growth inhibition in human colorectal carcinoma cells lacking the type V TGF-β receptor but expressing the type I and type II TGF-β receptors. These results suggest that the type V TGF-β receptor can mediate the TGF-β-induced growth inhibitory response in the absence of the type I or type II TGF-β receptor. These results also support the hypothesis that loss of the type V TGF-β receptor may contribute to the malignancy of certain carcinoma cells.

Transforming growth factor β (TGF-β)1 is the most potent polypeptide growth inhibitor for epithelial cells and plays an important role in the pathophysiology of epithelial cells in human and other species (1–3). The TGF-β-induced growth inhibition of epithelial cells is mediated by specific cell surface receptors (1–4). The type V TGF-β receptor is a 400-kDa nonproteoglycan membrane glycoprotein that co-expresses with the type I, type II, and type III TGF-β receptors in epithelial cells and other cell types but not in certain carcinoma cells (4, 5). The type V TGF-β receptor as well as the type I and type II TGF-β receptors are members of a new class of Ser/Thr-specific receptor protein kinases with distinct substrate specificities (6–11). The exact roles of these receptor kinases in the growth inhibition of epithelial cells induced by TGF-β are unknown. Recent studies have demonstrated that the heterocomplex formation of the type I and type II TGF-β receptors and the transphosphorylation of the type I TGF-β receptor by the type II TGF-β receptor are important in the TGF-β-induced growth inhibition (9–12). The role of the type V TGF-β receptor in the growth inhibition induced by TGF-β has not been defined. These studies reported here suggest that the type V TGF-β receptor forms heterocomplexes with the type I TGF-β receptor in mink lung epithelial cells and can mediate the TGF-β-induced growth inhibition in mink lung epithelial cells in the absence of the type I or type II TGF-β receptor. These studies reported here also support the hypothesis that loss of the type V TGF-β receptor may contribute to the malignancy of certain carcinoma cells.

EXPERIMENTAL PROCEDURES

Materials—Na235I (17 Ci/mg), [γ-32P]ATP (4,500 Ci/mmol), and Trans35S-label (1,000 Ci/mmol) were obtained from ICN Biochemicals, Inc. (Irvine, CA). [γ-32P]ATP was diluted with unlabeled ATP to have a specific radioactivity of ~10^4 cpm/pmol. Molecular mass protein standards (myosin, 205 kDa; β-galactosidase, 116 kDa; phosphorylase, 97 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 29 kDa), poly-L-lysine HBr (M, 15,000–30,000), β-mercaptoethanol, glycerol, Triton X-100, and other chemical reagents were obtained from Sigma. TGF-β1 was purchased from Austral Biologicals (San Ramon, CA). Disuccinimidyl suberate (DSS) was obtained from Pierce. Peptide antigen, a hexadecapeptide containing the ATP binding site amino acid sequence (6) was synthesized using tert-butoxycarbonyl chemistry on an Applied Biosystems model 431A peptide synthesizer. Recombinant human nonglycosylated insulin-like growth factor binding protein 3 (IGFBP-3) was provided by Celtrix Pharmaceuticals, Inc. (Santa Clara, CA). TGF-β receptor-defective mutants (DR26 and R1-B cells) and type II TGF-β receptor cDNA and neo-vector stably transfected hereditary human colorectal carcinoma cells (HII-37 and HCT 116 Neo cells) were provided by Drs. Joan Massague and Michael G. Brattain, respectively. Mink lung epithelial cells have been routinely maintained in the laboratory. All cultured cells were grown in 10% fetal calf serum in Dulbecco's modified Eagle's medium.

Preparation of Antiserum to the Type V TGF-β Receptor—The antiserum to the type V TGF-β receptor was raised in rabbits with the conjugate of bovine thyroglobulin and peptide antigen (a hexadecapeptide), whose amino acid sequence was derived from the ATP binding site amino acid sequence (6). The peptide antigen was conjugated to bovine thyroglobulin according to the procedure of Huang and Huang (13). The antiserum to the type V TGF-β receptor did not show reactivity to the type I and type II TGF-β receptors based on Western blot analysis and immunoprecipitation of 125I-TGF-β affinity labeled or Trans35S-label metabolically labeled type I and type II TGF-β receptors from mink lung epithelial cells in the presence of 0.1% SDS.

Purification of the Type V TGF-β Receptor from Bovine Liver Plasma Membranes—The type V TGF-β receptor was purified by DEAE-cellulose column chromatography after Triton X-100 extraction of bovine liver plasma membranes and wheat germ lectin-Sepharose 4B affinity
Type V TGF-β Receptor and Growth Inhibition

Immunoprecipitation of TGF-β1 Metabolically Labeled Mink Lung Epithelial Cells—Mink lung epithelial cells (Mv1Lu cells) were grown to confluence on P-60 Petri dishes and metabolically labeled with 35S-methionine according to the procedure of Huang and Huang (13). The monolayers were then treated with 0.1 nM TGF-β1 in Dulbecco’s modified Eagle’s medium, pH 7.4, at 0 °C for 30 min. The cells were then detached and lysed in 100 μl of 1% Triton X-100 in 10 mM Tris-HCl, pH 7.0, 125 mM NaCl, and 1 mM EDTA. After centrifugation, the Triton X-100 extracts were then diluted 10-fold with Triton X-100-free buffer and incubated with antisera or nonimmune serum (1:100 dilution) at 0 °C overnight. The immunocomplexes were precipitated with 20 μl of protein A-Sepharose (50%, v/v). After washing with 20 mM Tris-HCl, pH 7.4, 0.2% Triton X-100, the immunoprecipitates were analyzed by 6% SDS-polyacrylamide gel electrophoresis under reducing conditions and autoradiography. The relative intensity of 32P-labeled type V TGF-β receptor on the autoradiogram was quantitated by a PhosphorImager.

Afffinity Labeling of Mink Lung Epithelial Cells and Human Colorectal Carcinoma Cells—Mink lung epithelial cells (wild-type, DR26, and RIB cells) and hereditary human colorectal carcinoma cells (HCT 116 Neo and RII-37 cells) grown on P-60 Petri dishes were incubated with 125I-TGF-β1 (0.5, 1.5, and 1.5 mCi) in binding buffer (50 mM HEPES, pH 7.4, 128 mM NaCl, 5 mM KCl, 5 mM MgSO4, and 1.2 mM CaCl2) containing 0.2% bovine serum albumin at 0 °C for 2.5 h. The affinity labeling of cell surface TGF-β receptors was carried out as described previously (4, 5). The 125I-TGF-β1 affinity labeled receptors were then analyzed by 5.5% SDS-polyacrylamide gel electrophoresis under reducing conditions and autoradiography.

Immunocomplex Kinase Assay of the Type V TGF-β Receptor—The immunoprecipitation of the type V TGF-β receptor in mink lung epithelial cells treated with and without 0.1 nM TGF-β1, at 0 °C for 30 min, was carried out in 0.1% Triton X-100 as described above, except the cells were not metabolically labeled. The immunoprecipitates were incubated with 0.2 μM recombinant nonglycosylated human IGFBP-3 in 50 μl of 20 mM HEPES, pH 7.4, containing 10% glycerol, 0.1% Triton X-100, 5 mM (γ-32P)ATP, 0.1% mercaptoethanol, and 2.5 mM MnCl2. An aliquot of the reaction mixture was then analyzed by 7.5% SDS-polyacrylamide gel electrophoresis under reducing conditions and autoradiography. The relative intensity of 32P-IGFBP-3 on the autoradiogram was quantitated by a PhosphorImager.

RESULTS AND DISCUSSION

The type I, type II, and type III TGF-β receptors have been shown to form heterocomplexes upon ligand binding (12, 15–17). The formation of heterocomplexes appears to be important for initiation of signaling (type I and type II TGF-β receptor complex) (9–12, 15) or presentation of ligand (type III and type I/II TGF-β receptor complexes) (16, 17). To see whether the type V TGF-β receptor formed heterocomplexes with other TGF-β receptors, we performed immunoprecipitations of the type V TGF-β receptor and other TGF-β receptors using specific antisera to the type V TGF-β receptor. The specificity of the antisera to the type V TGF-β receptor had been validated by two types of evidence. First, the antisera reacted with the type V TGF-β receptor in Western blot analysis. Fig. 1 shows that the antisera specifically reacted with the type V TGF-β receptor in normal mink lung epithelial cells (Mv1Lu cells) that were metabolically labeled with 32Porthophosphate in response to TGF-β1 stimulation (Fig. 1B). The immunoprecipitated type V receptor exhibited a kinase activity toward IGFBP-3 (Fig. 1C). IGFBP-3 is a nonphysiological substrate for the type V TGF-β receptor but contains several SKE motifs that serve as the
the type V TGF-β receptors (Fig. 2). The arrowhead indicates the location of a 68-kDa protein, which is possibly an isoform or differentially glycosylated form of the type I TGF-β receptor. The arrow indicates the locations of the type I, type II, and type III TGF-β receptors (TβR). The arrow points to the location of the type V TGF-β receptor and dye front. B, mink lung epithelial cells were metabolically labeled with Trans-35S-label. The immunoprecipitations of the Triton X-100 and RIPA buffer extracts of the labeled cells, 5.5% SDS-polyacrylamide gel electrophoresis, and fluorography were carried out as described under “Experimental Procedures.” The arrow indicates the location of the type V and type I TGF-β receptors, and the bracket indicates the location of the type III TGF-β receptor. The arrowhead indicates the location of a 68-kDa protein, which is possibly an isoform or differentially glycosylated form of the type I TGF-β receptor.

Normal mink lung epithelial cells (Mv1Lu cells) have been commonly used to examine the cross-interaction of the TGF-β receptors and TGF-β-induced signal transduction. They express all the known TGF-β receptors (type I, type II, and type III) and TGF-β receptors and exhibit both growth inhibition and transcriptional activation in response to TGF-β stimulation (5, 18–20). In this study, the 125I-labeled TGF-β, affinity labeled cell surface receptors were immunoprecipitated with the antisera to the type V TGF-β receptor, after mink lung epithelial cells (Mv1Lu cells) were incubated with 0.1 nM 125I-TGF-β, for 2 1/2 h at 0°C, exposed to the cross-linking reagent DSS, and extracted with Triton X-100 buffer. The immunoprecipitates of the Triton X-100 buffer extracts were then analyzed by 5.5% SDS-polyacrylamide gel electrophoresis under reducing conditions and autoradiography. As shown in Fig. 2A, the type I TGF-β receptor was co-immunoprecipitated with the type V TGF-β receptor (Fig. 2A, lane 2). The immunoprecipitation efficiency for the type V TGF-β receptor was estimated to be approximately 30% of the total labeled receptor (Fig. 2A, lane 1). Excess peptide antigen blocked the immunoprecipitation of the TGF-β receptors (Fig. 2A, lane 3). Nonimmune serum did not immunoprecipitate the TGF-β receptors (Fig. 2A, lane 4). These results suggest that on the cell surface of mink lung epithelial cells, the type V TGF-β receptor forms heterocomplexes with the type I TGF-β receptor in the presence of the ligand.

To see whether the ligand was required for the heterocomplex formation, the TGF-β receptors were immunoprecipitated with antisera to the type V TGF-β receptor after mink lung epithelial cells (Mv1Lu cells) had been metabolically labeled with Trans-35S-label in the absence of exogenous ligand and extracted with Triton X-100 buffer or RIPA buffer (13). The immunoprecipitates of the Triton X-100 or RIPA buffer extracts were analyzed by 5.5% SDS-polyacrylamide gel electrophoresis under reducing conditions and autoradiography. As shown in Fig. 2B, the type V and type I (53 kDa) TGF-β

Fig. 2. Immunoprecipitation of TGF-β receptors with antisera to the type V TGF-β receptor after 125I-TGF-β, affinity labeling (A) and Trans-35S-label metabolic labeling (B) of mink lung epithelial cells. A, cell surface TGF-β receptors were affinity labeled with 125I-TGF-β and DSS in the presence (+) and the absence (−) of 100-fold molar excess of unlabeled TGF-β. The 125I-TGF-β, affinity labeled receptors were directly analyzed by 5.5% SDS-polyacrylamide gel electrophoresis under reducing conditions and autoradiography (lane 1) or extracted with Triton X-100 and immunoprecipitated with antisera (immune serum) to the type V TGF-β receptor in the absence (lane 2) and the presence (lane 3) of 0.5 μg/μl peptide antigen or with nonimmune serum (lane 4). The immunoprecipitates were then analyzed by 5.5% SDS-polyacrylamide gel electrophoresis and autoradiography. The brackets indicate the locations of the type I, type II, and type III TGF-β receptors (TβR). The arrows indicate the locations of the type V TGF-β receptor and dye front. B, mink lung epithelial cells were metabolically labeled with Trans-35S-label. The immunoprecipitations of the Triton X-100 and RIPA buffer extracts of the labeled cells, 5.5% SDS-polyacrylamide gel electrophoresis, and fluorography were carried out as described under “Experimental Procedures.” The arrow indicates the location of the type V and type I TGF-β receptors, and the bracket indicates the location of the type III TGF-β receptor. The arrowhead indicates the location of a 68-kDa protein, which is possibly an isoform or differentially glycosylated form of the type I TGF-β receptor.

Fig. 3. Identification of the type V TGF-β receptor in TGF-β receptor-defective mutants of mink lung epithelial cells (A and B) and growth inhibitory response of these mutants to TGF-β1 stimulation (C). A and B, cell surface TGF-β receptors were affinity labeled with 125I-TGF-β, (0.5, 1, and 1.5 nM) and DSS in type II and type I receptor-defective mutants (DR 26 and R-1B cells, respectively) and wild-type mink lung epithelial cells (Mv1Lu cells). The 125I-TGF-β, affinity labeled receptors were analyzed by 5% (panel A) or 5.5% (panel B) SDS-polyacrylamide gel electrophoresis under reducing conditions and autoradiography. The brackets indicate the locations of the type I, type II, and type III TGF-β receptors. The arrows indicate the locations of the type V TGF-β receptor and dye front. C, the TGF-β1-induced growth inhibition of mutants (DR 26 and R-1B cells) and wild-type cells (Mv1Lu cells) was assayed on 24-well clustered dishes based on the inhibition of [methyl-3H]thymidine incorporation into DNA of these cells stimulated by various concentrations of TGF-β1. The [methyl-3H]thymidine incorporation in wild-type cells without treatment with TGF-β1 was taken as 0% inhibition (51,000 ± 1, 500 cpm/well).
receptors were found in the immunoprecipitates of the Triton X-100 buffer extracts (Fig. 2B, lane 3), whereas only the type V TGF-β receptor was detected in the immunoprecipitates of the RIPA buffer extracts (Fig. 2B, lane 1). A 68-kDa protein was also identified in the immunoprecipitates of the Triton X-100 buffer extracts (Fig. 2B, lane 3). This protein was possibly an isoform or differentially glycosylated form of the type I TGF-β receptor because two different molecular mass isoforms of the type I TGF-β receptor were also identified in the 125I-TGF-β1 affinity labeling experiment (Fig. 2A, lanes 1 and 2). RIPA buffer contained 0.1% SDS that destabilized the heterocomplexes of the type V and type I TGF-β receptors. In the presence of RIPA buffer, only the type V TGF-β receptor was immunoprecipitated (Fig. 2B, lane 1). Because no endogenous TGF-β activity (growth inhibitory activity) was detected under the cultured conditions and because exogenous TGF-β1 did not affect the complexation of 35S-labeled type V and type I TGF-β receptors (data not shown), these results suggest that the type V TGF-β receptor can form heterocomplexes with the type I TGF-β receptor in the absence of ligand.

As described previously, mink lung epithelial cells (Mv1Lu cells) have been a useful system for studying the roles of the type I and type II TGF-β receptors in TGF-β-induced cellular responses. Mutant cells defective for the type I or type II TGF-β receptor have been reported to be unable to exhibit growth inhibition and transcriptional activation following TGF-β stimulation (18–21). However, the growth inhibition and transcriptional activation could be restored by genetic complementation between cells defective in the type I and type II TGF-β receptors (21). No study on the expression of the type V TGF-β receptor in these mutants has been reported. To test its presence, we investigated the expression of the type V TGF-β receptor in these mutants.

As shown in Fig. 3 (A and B), both the type II and type I TGF-β receptor-defective mutants (DR26 and R-1B cells, respectively) did express the type V TGF-β receptor, which was identified as the 400-kDa 125I-TGF-β1 affinity labeled protein. It is of importance to note that the type I TGF-β receptor-defective mutant (R-1B cells) expressed less of the type V TGF-β receptor when compared with the type II TGF-β receptor-defective mutant (DR26 cells).

Because both mutants (DR26 and R-1B cells) express the type V TGF-β receptor, an important role of the type V TGF-β receptor in TGF-β-induced cellular responses cannot be excluded in the genetic complementation experiments of the mutants (21). To determine whether the type V TGF-β receptor mediated a TGF-β-induced growth inhibition, we investigated the effect of higher concentrations of TGF-β1 on [methyl-3H]thymidine incorporation into DNA of mink lung epithelial cells (Fig. 3C). The K50 values for TGF-β1 or TGF-β2 binding to the type V receptor are approximately 20-fold higher than those for the type I and type II TGF-β receptors (6). If the type V TGF-β receptor can mediate a TGF-β-induced growth inhibition, TGF-β at higher concentrations should inhibit the DNA synthesis in the type I and type II TGF-β receptor-defective mutants. At $\sim$50–100 pM, TGF-β1 induced a small but significant inhibition of DNA synthesis (Fig. 3C) in both mutants ($\sim$20–40%). In type II TGF-β receptor-defective mutant cells (DR26 cells), the inhibition of DNA synthesis showed a small downward trend at $\sim$125 pM (Fig. 3C), but higher concentrations of TGF-β1 ($\sim$500 pM) did not cause a further decrease (30 ± 4% inhibition). A smaller degree of inhibition of DNA synthesis by TGF-β1 in type II TGF-β receptor-defective mutants was previously reported but was not discussed (20). These results suggest that the type V TGF-β receptor may mediate the growth inhibition in the absence of the type I or type II TGF-β receptor, although a higher level of ligand is required for the type V TGF-β receptor-mediated growth inhibition. In the wild-type cells (Mv1Lu cells) containing all TGF-β receptors, the maximal inhibition was observed at 1 pM TGF-β1.

In a previous study, we reported that several carcinoma cells lacked the type V TGF-β receptor and other TGF-β receptors (5). These cells lack the type V TGF-β receptor (MCF-7 and PC-12 cells) have been found not to respond to TGF-β stimulation with respect to growth inhibitory response (5). Recently, hereditary human colorectal carcinoma cells (HCT 116 cells)
were shown to be deficient in the type II TGF-β receptor (22). Stable transfection of these colorectal carcinoma cells with the type II TGF-β receptor cDNA was found to rescue the transcriptional response but failed to restore the growth inhibitory response to exogenous TGF-β (22). The inability of the transfected colorectal carcinoma cells to exert growth inhibitory response to TGF-β stimulation was also confirmed in our laboratory. One of the possibilities for the failure to restore the growth inhibitory response could be the lack of the type V TGF-β receptor expression in cells stably transfected with the neo-vector only (HCT 116 Neo cells) or with vector expressing the type II TGF-β receptor cDNA (RII-37 cells). To test this possibility, we performed the 125I-TGF-β affinity labeling of the TGF-β receptors in these cells. No detectable type V TGF-β receptor was found in these cells (Fig. 4, lanes 1–4). The type III and type II TGF-β receptors were detected in RII-37 cells (Fig. 4, lane 3). The type I TGF-β receptor migrated at the dye front and could not be identified in the 5% polyacrylamide system (Fig. 4, lane 3). Absence of the type V TGF-β receptor was confirmed by the observation that the type V TGF-β receptor antigen was not detected by Western blot analysis in HCT 116 Neo and RII-37 cells (data not shown). These results and the results from several experiments as shown in Fig. 3 can be summarized in Table I. The type V TGF-β appears to be critical for TGF-β-induced growth inhibition. The type I and type II TGF-β receptors are required for the maximal growth inhibition induced by TGF-β. Together with the observation that only transformed epithelial cells (carcinoma cells) have been found to lack the expression of detectable type V TGF-β receptor (5), these results also support the hypothesis that loss of the type V TGF-β receptor may contribute to the transformed state of certain carcinoma cells (e.g. hereditary human colorectal carcinoma cells) (5).

Together with the previous observations of distinct substrate specificities of the type V, type I, and type II TGF-β receptors (7, 23), the finding of heterocomplex formation of the type V TGF-β receptor and type I TGF-β receptor reported here raise an important question: What role does the type V TGF-β receptor play in various cellular responses induced by three different TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3)? The cellular responses induced by the TGF-β isoforms can vary substantially (1–3, 24, 25). The molecular mechanisms of the opposite effects of TGF-β1 versus TGF-β1 or TGF-β2 (25) are not easy to interpret within a model in which only the type I and type II TGF-β receptor heterocomplex mediates the signaling. The type V TGF-β receptor could be involved in these diverse cellular responses due to its distinct kinase substrate specificity (acidotrophic kinase activity versus the basic-trophic kinase activities of the type I and type II TGF-β receptors) (7, 23) and its different binding affinities to TGF-β isoforms. The distinct substrate specificity of the type V TGF-β receptor implies a different signaling pathway from those of the type I and type II TGF-β receptors. Segregation of growth inhibitory and transcriptional responses induced by TGF-β has been reported (22, 26, 27). The signaling pathway mediated by the type V TGF-β receptor could be important for growth inhibitory response but not obligatory for transcriptional response (the activation of transcription of fibronectin, collagen and plasminogen activator inhibitor-1 genes) (22). The cross-modulation of the two pathways mediated by the type VI and type II TGF-β receptors should be determined by the affinities of TGF-β isoforms to the TGF-β receptors and extracellular concentrations of TGF-β isoforms. The binding affinities of TGF-β1, TGF-β2, and TGF-β3 for the type I and type II TGF-β receptors are very similar with Kd of ~0.01 nM (24). The Kd of the type V TGF-β receptor for TGF-β1, TGF-β2, and TGF-β3 have been estimated to be ~0.4 nM, ~0.4 nM, and ~5 nM, respectively (7). Low concentrations of TGF-β3 might favor the formation of the type I and type II TGF-β receptor heterocomplexes that do not include the type V TGF-β receptor. Under the condition in which the amount of the type I receptor is limiting compared with those of other TGF-β receptors, TGF-β3 could alter the availability of the type I TGF-β receptor when the formation of type I and type V TGF-β receptor heterocomplex is required for certain cellular responses induced by TGF-β1 or TGF-β2.

Acknowledgments—We thank Dr. William S. Sly for critical review of the manuscript, Drs. Joan Massague and Michael G. Brattain for kindly providing TGF-β receptor-defective mutants (DR26 and R-I B cells) and type II TGF-β receptor cDNA and neo-vector transfected hereditary human colorectal carcinoma cells (HCT 116 cells), and Celtrix Pharmaceuticals, Inc., for providing recombinant human nonglycosylated IG-FBP-3. We also thank Tao Zhao for performing the kinase assay of the immuno precipitated type V receptor and Maggie Klevorn for preparing the manuscript.

REFERENCES
1. Moses, H. L., Yang, E. Y., and Pietenpol, J. A. (1991) Cell 63, 245–249
2. Roberts, A. B. (1991) in Peptide Growth Factors and Their Receptors (Sporn, M. B., and Roberts, A. B., eds) pp. 410–472, Springer-Verlag, Heidelberg, Germany
3. Massague, J. (1990) Annu. Rev. Cell Biol. 6, 597–641
4. O’Grady, P., Kuo, M.-D., Baldassare, J. J., Huang, S. S., and Huang, J. S. (1991) J. Biol. Chem. 266, 8583–8589
5. O’Grady, P., Huang, S. S., and Huang, J. S. (1991) Biochem. Biophys. Res. Commun. 179, 378–385
6. O’Grady, P., Liu, Q., Huang, S. S., and Huang, J. S. (1992) J. Biol. Chem. 267, 21035–21037
7. Liu, Q., Huang, S. S., and Huang, J. S. (1994) J. Biol. Chem. 269, 9221–9226
8. Lin, H. Y., Wang, X.-F., Ng-Enden, E., Weinberg, R. A., and Lodish, H. F. (1992) Cell 68, 775–785
9. Attisano, L., Carramico, J., Ventura, F., Weis, F. M. B., Massague, J., and Wrana, J. L. (1993) Cell 75, 671–680
10. Fonzi, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, Heldin, C.-H., and Miyazono, K. (1993) Cell 75, 681–692
11. Bassing, C. H., Yingling, J. M., Hewe, D. J., Wani, T., He, W. W., Gustafson, M. L., Shah, P., Donahoe, P. K., and Wang, X.-F. (1994) Science 263, 87–89
12. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massague, J. (1994) Nature 370, 341–347
13. Huang, S. S., and Huang, J. S. (1988) J. Biol. Chem. 263, 12608–12618
14. Lekshmik B. V., Huang, S. S., and Huang, J. S. (1990) J. Biol. Chem. 265, 1665–1675
15. Wrana, J. L., Attisano, L., Carramico, J., Zentella, A., Doody, J., Laiho, M., Wang, X.-F., and Massague, J. (1992) Cell 71, 1003–1014
16. López-Casillas, F., Wrana, J. L., and Massague, J. (1993) Cell 73, 1435–1444
17. Moustakas, A., Lin, H. Y., Henzi, Y. I., Flamondon, J., O’Connor-McCourt, M. D., and Lodish, H. F. (1993) J. Biol. Chem. 268, 22215–22218
18. Boyd, P. T., and Massague, J. (1989) J. Biol. Chem. 264, 2272–2278
19. Cheifetz, S., Hernandez, H., Laiho, M., ten Dijke, P., Iwata, K., and Massague, J. (1999) J. Biol. Chem. 265, 20533–20538
20. Laiho, M., Weis, F. M. B., and Massague, J. (1999) J. Biol. Chem. 265, 18518–18524
21. Laiho, M., Weis, F. M. B., Boyd, F. T., Ignatova, R. A., and Massague, J. (1999) J. Biol. Chem. 266, 9108–9112
22. Wang, J., Sun, L., Myeroff, L., Wang, X., Gentry, L. E., Yang, J., Liang, J., Zborowska, E., Markowitz, S., Wilson, J. K. V., and Brattain, M. G. (1995) J. Biol. Chem. 270, 22044–22049
23. Luo, K., Zhou, P., and Lodish, H. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11761–11765
24. Massague, J. (1992) Cell 69, 1067–1070
25. Shah, M., Foreman, D. M., and Ferguson, M. W. J. (1995) J. Cell Sci. 108, 985–1002
26. Geiger, A. G., Burmester, J. K., Webbink, R., Roberts, A. B., and Sporn, M. B. (1992) J. Biol. Chem. 267, 2588–2593
27. Ehner, R., Chen, R.-H., Lawler, S., Zionscheck, T., and Derynck, R. (1993) Science 262, 900–902