FKBP12 Is a Negative Regulator of Transforming Growth Factor-β Receptor Internalization*

(Received for publication, September 29, 1999, and in revised form, January 13, 2000)

Diyung Yao, Jules J. E. Doré, Jr., and Edward B. Leof‡
From the Thoracic Disease Research Unit and the Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, Minnesota 55905

Transforming growth factor-β (TGF-β) family polypeptides regulate cell growth and differentiation by binding to single pass serine/threonine kinases referred to as TGF-β type I and II receptors. Although interaction screens have shown that the immunophilin FKBP12 interacts with TGF-β type I receptors, the role of FKBP12 in TGF-β receptor action is presently unclear. Using a chimeric TGF-β receptor system, we have shown a specific enhancement of internalization when FKBP12 binding to the type I receptor was prevented with rapamycin. Moreover, although earlier studies demonstrated that type II receptor kinase activity was required for optimal internalization in mesenchymal cells, we found that rapamycin functioned downstream of the type II receptor kinase. Thus, rather than modulating TGF-β signaling, our data suggest a novel role for FKBP12 as a negative regulator of TGF-β receptor endocytosis.

The transforming growth factor-β (TGF-β) superfamily regulates a wide range of biological processes (1–3). A variety of structural and functional criteria have been used to group the superfamily into three classes consisting of TGFs-β, activins, and bone morphogenetic proteins. Members of these groups have been implicated in the regulation of wound healing, cellular proliferation, immune responses, and pattern formation throughout development.

Two distinct membrane receptor proteins referred to as TGF-β type I (TGF-βRI) and type II (TGF-βRII) receptors and a membrane-anchored proteoglycan called the type III receptor have been characterized and show high affinity TGF-β ligand binding (4–6). The type III receptor is proposed to present TGF-β to the signaling receptors and to enhance cell responsiveness to TGF-β; however, its short intracellular domain and absence of known signaling motifs suggest a limited role in direct regulation of TGF-β signaling. TGF-β signaling is primarily mediated by TGF-βRI and TGF-βRII. The signaling cascade begins when TGF-βRII binds ligand and recruits TGF-βRI into a heteromeric complex. TGF-βRII then transphosphorylates TGF-βRI at serine residues in the juxtamembrane glycine/serine-rich domain, and the activated TGF-βRI, in turn, interacts and activates downstream targets (7).

Interaction screens have demonstrated that the immunophilin FKBP12 binds to TGF-β type I receptors (8–10). FKBP12 belongs to a large family of peptidylprolyl cis-trans isomerases catalyzing the isomerization of peptidylprolyl imide bonds in peptide and protein substrates. It was first isolated as the receptor protein for the immunosuppressive drugs FK506 and rapamycin (11, 12). The FKBP12-FK506 complex inhibits calcineurin phosphatase activity and nuclear factor of activated T cells translocation, whereas the FKBP12-rapamycin complex inactivates TOR (target of rapamycin) family proteins (13, 14). Although the interaction of FKBP12 and the TGF-β type I receptor has been unequivocally established, its function in TGF-β-mediated signaling has been controversial. Initial reports proposed that FKBP12 functioned as a negative regulator of TGF-β signaling (15, 16). However, other studies reported no detectable effect on TGF-β signaling in FKBP12 null fibroblasts or by disrupting FKBP12/TGF-βRI binding with FK506 or rapamycin (17–19). It is presently unclear why such apparently conflicting results have been generated. Although a considerable amount of effort has been directed to studying the role of FKBP12 in TGF-β signaling, other possible functions of FKBP12 in regulating TGF-β activity have not been extensively investigated.

Our laboratory has developed a chimeric receptor system that employs the ligand-binding domain of the human GM-CSF α- or β-receptor fused to the TGF-β type I or II receptor transmembrane and cytoplasmic domains (20, 21). This model system permits one to determine the signaling and endocytic activities of defined TGF-β receptor complexes and provides a sensitive method to quantify the rates and extent of receptor internalization. In the course of studies identifying elements within the chimeric type I receptor that control receptor trafficking, we noted the presence of a potential negative regulator of endocytosis near the chimeric type I receptor glycine/serine-rich domain (amino acids 185–192). Since FKBP12 binds to a nearby Leu-Pro sequence at amino acids 193 and 194 (17), we investigated whether FKBP12 binding to the chimeric type I receptor might be inhibitory to TGF-β receptor internalization.

In this report, we observed a specific enhancement of chimeric TGF-β receptor internalization when FKBP12 binding to the chimeric type I receptor was prevented. This effect occurred independently of type II receptor kinase activity. We also found that FKBP12 does not modulate basal or chimeric TGF-β receptor-mediated induction of plasminogen activator inhibitor-1 (PAI-1) or fibronectin proteins in fibroblast cell lines. As such, our data suggest that a novel physiological function of FKBP12 is to act as a negative regulator of TGF-β receptor endocytosis.

* This work was supported by National Institutes of Health Grants GM54200 and GM55816 and by the Mayo Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed: Guggenheim 6, Mayo Clinic, Rochester, MN 55905. Tel.: 507-284-5717; Fax: 507-284-4521; E-mail: leof.edward@mayo.edu.
* The abbreviations used are: TGF-β, transforming growth factor-β; TGF-βRI, TGF-β receptor; GM-CSF, granulocyte/macrophage colony-stimulating factor; PAI-1, plasminogen activator inhibitor-1; EGF, epidermal growth factor; FBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline.

This paper is available on line at http://www.jbc.org

1 The abbreviations used are: TGF-β, transforming growth factor-β; TGF-βRI, TGF-β receptor; GM-CSF, granulocyte/macrophage colony-stimulating factor; PAI-1, plasminogen activator inhibitor-1; EGF, epidermal growth factor; FBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline.
EXPERIMENTAL PROCEDURES

Materials—

125I-Labeled recombinant human GM-CSF and epidermal growth factor (EGF) were purchased from NEN Life Science Products. To document activation of endogenous TGF-β receptors, TGF-β1 and TGF-β2 were purchased from Austral Biologicals (San Ramon, CA) or R&D Systems (Minneapolis, MN); no difference in activity was observed for either isoform (data not shown). Rapamycin was purchased from Sigma.

Cell Culture—Chimeric receptor-expressing fibroblast clones were maintained in 5% FBS/DMEM containing 100 μg/ml bioactive Genetin (Life Technologies, Inc.) and 50 μg/ml hygromycin B (Roche Molecular Biochemicals) as described (20).

Isolation of Clones—Parental AKR-2B cells were plated at 1 × 104 cells/well in six-well plates 24 h prior to transfection. Cells were transfected with serum-free DME and then incubated for 8 h in 2 ml of DME with transfection solution consisting of 2–4 μg of expression plasmid DNA and 2 μg/ml TransIT™ LT2 (PanVera Corp., Madison, WI) in a final volume of 100 μl with Opti-MEM (Life Technologies, Inc.). Cells were allowed to recover for 16 h in 5% FBS/DMEM and then placed in selective medium (5% FBS/DME with 600 μg/ml Genetin and 350 μg/ml hygromycin B) for 24 h before splitting 1:40 by surface area. Separated colonies were isolated and expanded after 2–3 weeks.

Receptor Mutagenesis—L193A/P194A mutant constructs were made using the QuikChange™ site-directed mutagenesis kit (Stratagene). The polymerase chain reaction template was the chimeric type I receptor consisting of the extracellular domain of the GM-CSF receptor α-subunit and the transmembrane and intracellular domains of the TGF-β type 1 receptor (20). The primers used for amplification were 5′-CCGCAAGCTTACgcGCTGTTCAGAGA and 3′-TCTCTGAACAAAGcGTCGGAACACCAG (lowercase letters indicate the mutated nucleotides). The polymerase chain reaction was set up at 94 °C for 1 min, 50 °C for 2 min, and 65 °C for 18 min and was repeated for 18 cycles. The insert was verified by automated DNA sequencing and subcloned into the mammalian expression vector pNa (20).

Internalization Assays—Cells were seeded at 1 × 104 cells/well in six-well plates (16–24 h prior to the experiment) in 5% FBS/DMEM. Ligand binding was performed at 4 °C in binding buffer (DME containing 200 mM HEPES, pH 7.4, and 25 mM mg/ml bovine serum albumin) supplemented with 100 μCi 125I-labeled GM-CSF in the presence or absence of a 20-fold molar excess of unlabeled GM-CSF to document specific binding. Once equilibrium had been reached (2–4 h), the plates were washed twice with binding buffer. 1 ml of 5% FBS/DMEM (prewarmed to 37 °C) with or without 10 μM rapamycin was added to each well (final ethanol concentration of 0.1% for all wells), and the plates were returned to 37 °C for the indicated times. Percent internalization (i.e. specific cpm in cell/specific surface cpm) was determined after acid washing (PBS, pH 3.0; two 2-min washes) to remove surface-bound ligand and cell lysis in 0.2M NaOH and 40 μg/ml salmon sperm DNA. EGF internalization was measured as described for GM-CSF binding. As shown in Fig. 1, defined as the probability of an occupied receptor being internalized in 1 min at 37 °C, represents the slope of the line when the ratio of the internalized versus surface-bound ligand is plotted as a function of time. This constant is independent of receptor affinity or number and has been shown to be an accurate reflection of internalization (22).

Degradation of 125I-Labeled GM-CSF Ligand—Cells were seeded at 1.5 × 105 cells/well in six-well plates the day before the experiment. Plates were cooled at 4 °C for 20 min, followed by two washes with ice-cold binding buffer. 100 μCi 125I-labeled GM-CSF in the presence or absence of a 20-fold molar excess of unlabeled GM-CSF in binding buffer was added to each well, and the plates were rocked at 4 °C for 2 h. The cells were washed with 75% calf serum and 25% binding buffer, and 1 ml of 5% FBS/DMEM (prewarmed to 37 °C) with or without 10 μM rapamycin was added to each well. At the indicated times, the medium was removed and replaced with 50 μl of trichloroacetic acid. The cells were solubilized with 0.2 × NaOH and 40 μg/ml salmon sperm DNA to determine cell-associated counts. Preliminary experiments demonstrated that degraded ligand was almost exclusively secreted into the medium (data not shown). The extent of degradation was measured as the tri-chloroacetic acid-soluble counts divided by the sum of the two fractions.

PAI-1 Production—Cells were seeded in six-well plates at 1.5 × 105 cells/well 24 h before the experiment. The next day, the growth medium was removed, and cells were washed twice with 1× PBS. 1 ml of methionine-free DMEM alone or containing 10 ng/ml either GM-CSF or TGF-β2 was added to each well, and the plates were incubated at 37 °C. Cells were then pulsed for 2 h with 50 μCi of Easy Tag EXPRESS [35S] protein labeling mixture (NEN Life Science Products) and processed by washing (on ice) once with 1× PBS; three times with 10 mM Tris, pH 8.0, 0.5% deoxycholate, and 50 μg/ml phenylmethylsulfonyl fluoride; twice with 2 mM Tris, pH 8.0; and once with 1× PBS (23). The remaining matrix proteins were eluted from the plate by addition of 100 μl of 2× Laemmli buffer containing 10% β-mercaptoethanol. The samples were separated by 8% SDS-polyacrylamide gel electrophoresis, followed by fluorography.

Fibronectin Production—Cells were plated at 2 × 105 cells/well in six-well plates and incubated overnight. The next day, the cells were washed twice with 1× PBS and placed in serum-free MCDB 402 medium (JRH Biosciences) for 24 h. The following day, the growth medium was replaced with 1 ml of methionine-free DMEM alone (control) or containing 10 ng/ml either GM-CSF or TGF-β2 in the presence of 50 μCi of Easy Tag EXPRESS [35S] protein-labeling mixture. The plates were returned to the incubator for 4 h. The medium was collected and mixed with 50 μl of gelatin-Sepharose (Amersham Pharamacia Biotech) and 50 μl of 10% Triton X-100 and rocked at 4 °C for 2 h. Following washing once with Tris-buffered saline (50 mM Tris and 150 mM NaCl), once with 50 mM Tris and 500 mM NaCl, and again with Tris-buffered saline, the bound proteins were eluted with 50 μl of 2× Laemmli buffer containing 10% β-mercaptoethanol. The samples were separated by 6% SDS-polyacrylamide gel electrophoresis, followed by fluorography.

RESULTS

Rapamycin Specifically Promotes Internalization of Chimeric TGF-β Receptors—The A105 clone is an AKR-2B mouse fibroblast cell line that expresses wild-type chimeric TGF-β type I and type II receptors. Addition of GM-CSF activates both the signaling and endocytic activities of the receptor complex (20, 24). In the course of studies using chimeric type I receptor truncation mutants, we noted the presence of a potential negative regulator of endocytosis acting within/nearby the glycine/serine-rich domain. Since this region overlaps the FKBPI2-binding site and because the role of FKBPI2 in TGF-β receptor action has been controversial, we decided to examine the effect of FKBPI2 binding on chimeric receptor internalization.

125I-Labeled GM-CSF binding was performed at 4 °C, and the cultures were shifted to 37 °C in the absence or presence of 10 μM rapamycin to sequester intracellular FKBPI2 (8). As shown in Fig. 1, addition of rapamycin significantly enhanced chimeric receptor internalization (p < 0.05). Moreover, similar results were obtained using mink lung epithelial cells or by substituting FK506 to prevent FKBPI2 binding to the chimeric type I receptor (data not shown). Thus, agents that sequester FKBPI2 enhanced chimeric TGF-β receptor internalization, and the effects were observed in both mesenchymal and epithelial cells.

Since rapamycin is a potent immunosuppressant with a variety of cellular effects (14), we were concerned about the specificity of the response to chimeric TGF-β receptor internalization. To address this question, we determined whether rapamycin would modulate internalization of the EGF receptor also expressed on A105 cells. The EGF receptor system was chosen because it is a well characterized model for receptor-mediated endocytosis, and it has not been reported to bind FKBPI2. As shown in Fig. 1 (inset), although the kinetics of EGF receptor internalization were significantly faster than those of chimeric TGF-β receptor internalization (24, 25), no significant differences were found with EGFP receptor internalization following rapamycin treatment. In fact, at 15 min, the cells treated with rapamycin had undergone internalization to an even lesser (not greater, as seen with TGF-β receptors) extent than the cells treated with vehicle alone. These results are consistent with the hypothesis that rapamycin specifically enhances chimeric TGF-β receptor internalization through se-
FKBP12 binding is mediated through a Leu-Pro motif within the TGF-β type I receptor at amino acids 193 and 194 (28). Mutation of these two residues to alanines has been shown to abolish the ability of TGF-βRI to bind FKBP12 (17). If the enhanced internalization seen in Figs. 1 and 2A occurred through a specific effect of rapamycin in preventing FKBP12/TGF-βRI binding, then mutation of the FKBP12-binding site should abrogate the response to rapamycin. To that end, we established several stable fibroblast lines harboring the L193A/P194A double mutations in the chimeric type I receptor (coexpressed with the wild-type chimeric type II receptor) and tested them for the ability to internalize 125I-labeled GM-CSF in the absence and presence of rapamycin. As shown in Fig. 3, rapamycin did not significantly enhance ligand internalization when the FKBP12-binding site in the chimeric type I receptor was eliminated. These results (Figs. 1, 2A, and 3) indicate that the increase in chimeric TGF-β receptor internalization by rapamycin is specifically mediated through FKBP12 and suggest that FKBP12 functions as a negative regulator of TGF-β receptor internalization.

**Mutant Type I Receptors (L193A/P194A) Deficient in TGF-β Binding Do Not Exhibit Enhanced TGF-β Signaling**—As discussed previously, the role of FKBP12 in TGF-β signaling is controversial (15–19). It was therefore of interest to examine the signaling activity of the chimeric type I receptor deficient in FKBP12 binding (Fig. 4). Stable fibroblast clones expressing mutant chimeric TGF-βRI (L193A/P194A) and wild-type chimeric TGF-βRII were examined for ligand-stimulated secretion of PAI-1 and fibronectin proteins. As shown in Fig. 4 (A and B), the parental A105 cells exhibited ligand-dependent induction of PAI-1 and fibronectin proteins when stimulated with either GM-CSF (activates chimeric TGF-β receptors) or TGF-β2 (activates endogenous TGF-β receptors). Moreover, L193A/P194A receptor-expressing clones also responded to treatment with GM-CSF or TGF-β2 by increasing the expression of both PAI-1 and fibronectin proteins. Although there were quantitative differences as compared with A105 cells, neither the basal nor the induced secretion of PAI-1 and fibronectin proteins was elevated by the L193A/P194A mutation. Thus, altering the FKBP12-binding site in the chimeric TGF-β type I receptor did not enhance receptor-mediated signaling activities.

**FKBP12 Regulates TGF-β Type II Receptor Internalization**—Previous studies in our laboratory have demonstrated that the kinase activity of TGF-βRII is required for optimal ligand internalization in mesenchymal cells (21). It has also been shown that FKBP12 is gradually released from TGF-βRII once the receptor is phosphorylated by TGF-βRII (15, 16). We therefore hypothesized that in addition to its obligate signaling role, an endocytic role of the type II receptor kinase is to function as a mechanism to disassociate the negative regulator FKBP12 from the type I receptor. As such, a direct extension of this hypothesis would be that rapamycin should abrogate the type II receptor kinase requirement for internalization via sequestering FKBP12 from the type I receptor. To test this possibility, we utilized several lines expressing wild-type chimeric type I receptors and mutant chimeric type II receptors harboring a mutation in the presumptive ATP-binding site (K277R) and investigated their response to rapamycin. These clones had been previously reported to have a diminished ability to internalize ligand and signal through the chimeric receptors (21). As shown in Fig. 5A, addition of rapamycin enhanced internalization of the K277R type II receptor by ~35%, indicating an action downstream of the receptor kinase.

The TGF-β type II receptor has both trans- as well as auto-
phosphorylation activities (7). Although the K277R mutation will abolish both responses, a mutation at proline 525 to leucine (P525L) was shown to generate a type II receptor capable of autophosphorylation, yet lacking the ability to transphosphorylate an associated type I receptor (29). This not only generated a receptor complex unable to signal, but one with diminished endocytic activity (21). As such, if TGF-βRI phosphorylation (presumably by TGF-βRII) was required for the release of FKBP12, and rapamycin enhanced chimeric receptor internalization via dissociating FKBP12 from the type I receptor, the P525L mutant receptor should also show enhanced internalization with rapamycin similar to the K277R receptor. Fig. 5B shows that this did in fact occur. Thus, rapamycin enhances internalization of chimeric TGF-β receptors in the absence of type II receptor kinase activity.

**DISCUSSION**

FKBP12 is an abundant and ubiquitously expressed protein that catalyzes peptidylprolyl cis-trans isomerization (11, 12). It
FKBP12 Regulates TGF-β Receptor Internalization

is also classified as an immunophilin due to its ability to bind and activate the immunosuppressive activity of the macrolides FK506 and rapamycin. Although it has been shown convincingly that (i) FKBP12 associates with the TGF-β type I receptor, (ii) both FK506 and rapamycin effectively compete with the type I receptor for FKBP12, and (iii) type I receptor phosphorylation results in the gradual release of FKBP12 in a ligand-dependent manner, the physiological role of FKBP12 in TGF-β receptor signaling is still controversial (8, 15–19). In the course of studies designed to identify elements in the type I receptor controlling chimeric receptor trafficking, we noted that a negative regulator of endocytosis acted near the glycine-serine-rich domain. Since FKBP12 is the only protein known to bind the type I receptor in this region, we investigated the possibility of whether FKBP12 regulated TGF-β receptor internalization.

To address the possible role of FKBP12 in TGF-β receptor endocytosis, we have employed our previously characterized chimeric receptor-expressing cultures (20, 24). Using this system, we found that rapamycin enhanced the internalization of chimeric TGF-β receptors in both AKR-2B mouse fibroblasts (Fig. 1 and 2A) and Mv1Lu mink lung epithelial cells (data not shown). Interestingly, although the rate of 125I-labeled GM-CSF internalization was increased in the presence of rapamycin, a corresponding increase in ligand degradation was not observed (Figs. 1 and 2B). A similar dissociation of internalization and degradation has been reported by Dhanwada et al. (26) and Bradbury and Menon (27) in studies of mutant luteinizing hormone receptors. Although a mechanism has not been defined, both our results might be a reflection of the differences in the kinetics of the internalization and degradation processes. For instance, following steady-state binding, the rapid rate of ligand internalization might saturate the intracellular sorting machinery (30, 31). As such, since endocytic sorting proceeds degradation, increased internalization would not necessarily lead to increased degradation. To that end, a current focus of our laboratory is to define the subcellular compartment(s) and effector(s) regulating TGF-β trafficking.

Since rapamycin has numerous effects on cultured cells (14), it was necessary to document that the increase in ligand internalization was specific to the TGF-β receptor system. This concern was initially addressed by examining EGF receptor internalization. The data demonstrated that rapamycin did not enhance the internalization of EGF receptors (Fig. 1, inset), which also internalize ligand through a clathrin-dependent mechanism, but have not been reported to interact with FKBP12 (24, 25). This implies that the action of rapamycin in promoting chimeric TGF-β receptor internalization is not a nonspecific result of drug treatment, but is likely mediated through FKBP12. To investigate that hypothesis further, mutant type I receptors (L193A/P194A) incapable of binding FKBP12 were generated (28). If the enhanced internalization by rapamycin observed in Fig. 1 was a result of releasing FKBP12 from the type I receptor, then the L193A/P194A receptors should not be affected by rapamycin treatment. As shown in Fig. 3, the L193A/P194A mutants showed no statistically significant differences in rates and/or extent of internalization in the presence and absence of rapamycin. Moreover, although the extent of internalization of the L193A/P194A mutant was less than that of the wild-type receptors (compare Figs. 1 and 3), which is likely due to both differential receptor expression as well as clonal variation, the rate constant for the L193A/P194A mutant (in the absence of rapamycin) was in fact slightly higher than that for the wild type (0.063 versus 0.048 min⁻¹, respectively). This is consistent with the hypothesis that through binding to the type I receptor, FKBP12 functions as a negative regulator of TGF-β receptor endocytosis.

The kinase activity of the type II receptor provides an obligate function for optimal TGF-β receptor internalization in AKR-2B fibroblasts (21). Furthermore, additional studies have shown that FKBP12 is slowly released from the type I receptor
upon ligand stimulation and type I receptor phosphorylation (15, 16). We thus hypothesized that besides phosphorylating the type I receptor to initiate the signaling cascade, the type II receptor kinase is also able to enhance receptor internalization via mechanisms such as promoting the dissociation of FKBP12, a negative regulator of internalization. This hypothesis prompted the studies shown in Fig. 5, where TGF-β receptor internalization was tested in cell lines expressing mutant chimeric type II receptors (K277R and P525L) unable to phosphorylate an associated type I receptor. The results show that rapamycin significantly enhanced internalization in the absence of chimeric type II receptor kinase activity. Although the extent of internalization was less than that observed for wild-type chimeric receptors (Fig. 1), indicating either multiple functions of the receptor kinase or clonal variation in internalization rates, the data support an action of rapamycin downstream of the type II receptor kinase.

To date, the physiological role(s) of FK506-binding proteins remains elusive. They have been implicated in assisting protein folding and immunosuppression and as components of multisubunit assemblies (11, 12). For example, FKBP12 was demonstrated to associate with the inositol 1,4,5-triphosphate receptor and the release of FKBP12 by treatment with FK506 or rapamycin enhanced spontaneous calcium efflux and sensitivity to inositol 1,4,5-triphosphate (32). It was thus proposed that the primary purpose of this family of peptidylprolyl cis-trans isomerases is to interact with a proline residue and to hold the peptidylprolyl bond in a transition state to modulate protein folding and immunosuppression and as components of multisubunit assemblies (11, 12).

REFERENCES

1. Moses, H. L., and Serra, R. (1996) *Curr. Opin. Genet. Dev.* 6, 581–586
2. Massague, J. (1990) *Cell* 65, 847–856
3. Kingsley, D. M. (1994) *Genes Dev.* 8, 133–146
4. Franzen, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Heldin, C. H., and Miyazono, K. (1995) *Cell* 75, 781–792
5. Lin, H. Y., Wang, X. F., Ng, E. E., Weinberg, R. A., and Lodish, H. F. (1992) *Cell* 68, 775–785
6. Wang, X. F., Lin, H. Y., Ng, E. E., Downward, J., Lodish, H. F., and Weinberg, R. A. (1991) *Cell* 67, 797–805
7. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massague, J. (1994) *Nature* 370, 341–347
8. Wang, T. W., Donahoe, P. K., and Zervos, A. S. (1994) *Science* 265, 674–676
9. Chang, M. J., Zhang, D., Kinnunen, P., and Schneider, M. D. (1998) *J. Biol. Chem.* 273, 9365–9368
10. Huse, M., Chen, Y. G., Massagué, J., and Kuriyan, J. (1999) *Cell* 96, 425–436
11. Göthel, S. F., and Marahiel, M. A. (1999) *Cell. Mol. Life Sci.* 55, 423–436
12. Kay, J. E. (1996) *Biochem. J.* 314, 361–385
13. Liu, J., Farmer, J. D. J., Lane, W. S., Friedman, J., Weissman, I., and Schreiber, S. L. (1991) *Cell* 66, 807–815
14. Abraham, R. T., and Wiederrecht, G. J. (1996) *Annu. Rev. Immunol.* 14, 483–510
15. Chen, Y. G., Lin, F., and Massagué, J. (1997) *EMBO J.* 16, 3866–3876
16. Wang, T. W., Li, B. Y., Danielson, P. D., Shah, P. C., Rockwell, S., Lechleider, R. J., Martin, J., Manganaro, T., and Donahoe, P. K. (1996) *Cell* 86, 435–444
17. Charng, M. J., Kinnunen, P., Hawker, J., Brand, T., and Schneider, M. D. (1999) *J. Biol. Chem.* 274, 22941–22944
18. Bassing, C. H., Shou, W., Muir, S., Heitman, J., Matzuk, M. M., and Wang, T. W. (1998) *Cell Growth Differ.* 9, 223–228
19. Shou, W., Aghdasi, B., Armstrong, D. L., Guo, Q., Bao, S., Chang, M. J., Mathews, L. M., Schneider, M. D., Hamilton, S. L., and Matzuk, M. M. (1998) *Nature* 391, 489–492
20. Anders, R. A., and Leof, E. B. (1996) *J. Biol. Chem.* 271, 21758–21766
21. Anders, R. A., Dore, J. E. Jr., Arline, S. L., Garamszegi, N., and Leof, E. B. (1998) *J. Biol. Chem.* 273, 23118–23125
22. Anders, R. A., and Leof, E. B. (1997) *Cell Growth Differ.* 8, 2133–2143
23. Sorkin, A., and Water, C. M. (1993) *Bioessays* 15, 375–382
24. Dhanwada, K. R., Vijapurkar, U., and Ascoli, M. (1996) *Mol. Endocrinol.* 10, 544–554
25. Bradbury, F. A., and Menon, K. M. J. (1999) *Biochemistry* 38, 8703–8712
26. Okadome, T., Oeda, E., Saitoh, M., Hidemori, I., Moses, H., Miyazono, K., and Kawahata, M. (1996) *J. Biol. Chem.* 271, 21687–21690
27. Cassano, J., Zentella, A., and Massague, J. (1995) *Mol. Biol. Cell.* 15, 1573–1581
28. Marks, M. S., Woodruff, L., Ohno, H., and Bonifacino, J. S. (1996) *J. Cell Biol.* 135, 341–354
29. Warren, R. A., Green, F. A., Stenberg, P. E., and Enns, C. A. (1998) *J. Biol. Chem.* 273, 17056–17063
30. Cameron, A. M., Steiner, J. P., Sabatini, D. M., Kaplan, A. I., Walesky, L. D., and Snyder, S. H. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 1784–1788
FKBP12 Is a Negative Regulator of Transforming Growth Factor-β Receptor Internalization
Diying Yao, Jules J. E. Doré, Jr. and Edward B. Leof

J. Biol. Chem. 2000, 275:13149-13154.
doi: 10.1074/jbc.275.17.13149

Access the most updated version of this article at http://www.jbc.org/content/275/17/13149

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 17 of which can be accessed free at http://www.jbc.org/content/275/17/13149.full.html#ref-list-1