The Protein-tyrosine Phosphatase TCPTP Regulates Epidermal Growth Factor Receptor-mediated and Phosphatidylinositol 3-Kinase-dependent Signaling*

(Received for publication, May 5, 1999, and in revised form, July 12, 1999)

Tony Tiganis‡§, Bruce E. Kemp‡, and Nicholas K. Tonks‡§

From ‡St. Vincent’s Institute of Medical Research, Fitzroy, Victoria 3065, Australia and §Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

In this study we have investigated the down-regulation of epidermal growth factor (EGF) receptor signaling by protein-tyrosine phosphatases (PTPs) in COS1 cells. The 45-kDa variant of the PTP TCPTP (TC45) exits the nucleus upon EGF receptor activation and recognizes the EGF receptor as a cellular substrate. We report that TC45 inhibits the EGF-dependent activation of the c-Jun N-terminal kinase, but does not alter the activation of extracellular signal-regulated kinase 2. These data demonstrate that TC45 can regulate selectively mitogen-activated protein kinase signaling pathways emanating from the EGF receptor. In EGF receptor-mediated signaling, the protein kinase PKB/Akt and the mitogen-activated protein kinase c-Jun N-terminal kinase, but not extracellular signal-regulated kinase 2, function downstream of the phosphatidylinositol 3-kinase (PI 3-kinase). We have found that TC45 and the TC45-D182A mutant, which is capable of forming stable complexes with TC45 substrates, inhibit almost completely the EGF-dependent activation of PI 3-kinase and PKB/Akt. TC45 and TC45-D182A act upstream of PI 3-kinase, most likely by inhibiting the recruitment of the p85 regulatory subunit of PI 3-kinase by the EGF receptor. Recent studies have indicated that the EGF receptor can be activated in the absence of EGF following integrin ligation. We find that the integrin-mediated activation of PKB/Akt in COS1 cells is abrogated by the specific EGF receptor protein-tyrosine kinase inhibitor tyrphostin AG1478, and that TC45 and TC45-D182A can inhibit activation of PKB/Akt following the attachment of COS1 cells to fibronectin. Thus, TC45 may serve as a negative regulator of growth factor or integrin-induced, EGF receptor-mediated PI 3-kinase signaling.

Protein-tyrosine phosphatases (PTPs) are large and structurally diverse family of enzymes, characterized by the consensus sequence (IV)XGXXXR. They are found in eukaryotes, prokaryotes and viruses and can either antagonize or potentiate protein-tyrosine kinase (PTK)-dependent signaling. PTPs have been shown to participate as either positive or negative regulators of signal transduction in a wide range of physiological processes, which include cellular growth and proliferation, migration, differentiation and survival (1–3). Despite their important roles in such fundamental physiological processes, the mechanism by which PTPs exert their effects is often poorly understood.

The human T-Cell PTP (TCPTP) is an intracellular non-transmembrane phosphatase that was originally cloned from a T-cell cDNA library but is now known to be expressed in many tissues. TCPTP contains a conserved catalytic domain and a non-catalytic C-terminal segment that varies in size and function as a result of alternative splicing. Two splice variants differing only in their extreme C termini are expressed. The 48-kDa form of human TCPTP (TC48) contains a 34-residue hydrophobic tail, which is replaced by a hydrophilic 6-residue sequence in the 45-kDa form (TC45). TC48 localizes to the endoplasmic reticulum (ER) (4, 5), whereas under basal conditions TC45 is localized in the nucleus due to the presence of a bipartite nuclear localization sequence (5–9).

All PTPs contain an aspartic acid that is essential for catalysis. Mutation of this residue, Asp-182 in TCPTP, to alanine reduces the catalytic activity but maintains a high affinity for substrates, thereby generating a “substrate trapping” mutant, which can form stable complexes with tyrosine-phosphorylated proteins in vitro (10) and in vivo (11, 12). Using the TCPTP D182A substrate trapping mutants, we have shown previously that TCPTP displays a restricted specificity in a cellular context, and that the EGF receptor is one of its substrates (12). Both TC48 and TC45 recognize the tyrosine-phosphorylated EGF receptor as a substrate in a cellular context; TC48 recognizes the receptor as it proceeds through the ER and may function to prevent inappropriate signaling by the nascent receptor during synthesis, whereas TC45 can exit the nucleus in response to EGF and gain access to signaling complexes containing the EGF receptor at the plasma membrane (12).

In the present study we have examined the effect of overexpression of TC45 on EGF receptor-induced signaling events. We show that TC45 can inhibit the EGF-induced activation of PKB/Akt and that this correlates with a reduced association of PI 3-kinase with the activated EGF receptor. In addition, we show that plating of COS1 cells on fibronectin leads to activation of PKB/Akt, in an EGF-independent but EGF receptor and PI 3-kinase-dependent manner, which is also inhibited by TC45. Thus, TC45 may serve as a negative regulator of specific signals from the EGF receptor that mediate PKB/Akt activation.

* This work was supported in part by National Institutes of Health Grant CA53840 (to N. K. T.) and by grants from the National Health and Medical Research Council of Australia (to T. T. and B. E. K.). 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.

§ National Health and Medical Research Council of Australia. J. Martin Fellow. To whom correspondence should be addressed: St. Vincent’s Inst. of Medical Research, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia. Tel.: 61-3-9288-2480; Fax: 61-3-9416-2676; E-mail: tiganis@ariel.ucs.unimelb.edu.au.

‡ The abbreviations used are: PTP, protein-tyrosine phosphatase; PTK, protein-tyrosine kinase; TCPTP, T-cell PTP; ER, endoplasmic reticulum; EGF, epidermal growth factor; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis; FBS, fetal bovine serum; BSA, bovine serum albumin; PKB, phosphoinositide 3-kinase B; DNAM, Dulbecco’s modified Eagle’s medium; SH, Src homology; ERK, extracellular signal-regulated kinase; PKB, protein kinase B; JNK, c-Jun N-terminal kinase; PI, phosphatidylinositol; GST, glutathione S-transferase.

© 1999 by The American Society for Biochemistry and Molecular Biology, Inc.

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

27768
**Materials**

Recombinant human EGF was purchased from Genzyme Diagnostics (Cambridge, MA), human plasma fibronectin from Life Technologies, Inc., wortmannin and crude brain lipids were from Sigma. Monoclonal EGF receptor Ab-1 antibody used for immunoprecipitation was purchased from Calbiochem Oncogene Research Products (Cambridge, MA), polyclonal EGF receptor (100S) antibody used for immunoblotting from Santa Cruz Biotechnology (Santa Cruz, CA). PI 3-kinase p85 (P13020) antibody from Transduction Laboratories (Lexington, KY), phospho-Akt (Ser-473) and Akt antibodies from New England Biolabs (Beverly, MA), and FLAG M2 antibody from Eastman Kodak Co. The following constructs and reagents were generously provided by colleagues: hemagglutinin-tagged (HA)-PKB/Akt pCECE by B. Hemmings (Friedrich Miescher Institut, Basel, Switzerland), p110K227E pS505 by J. Downward (Imperial Cancer Research Fund, London, United Kingdom), FLAG-JNK by L. V. Aventel (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), HA-ERK2 pJ3H by J. Chernoff (Temple University, Philadelphia, PA), monoclonal ERK2 1B3B9 antibody by M. Weber (University of Virginia, Charlottesville, VA), monoclonal anti-TCPTP antibody CP4 by D. Hill (Calbiochem Oncogene Research Products, Cambridge, MA), and tyrphostin AGY78 by T. Thompson (St. Vincent’s Institute of Medical Research, Melbourne, Australia). Monoclonal anti-phosphotyrosine antibodies G9 (sub type IgM) and G104 (sub type IgG) have been described previously (10, 12).

**Cell Culture, Transfections, and Electroporations**

COS1 cells were cultured at 37 °C and 5% CO2 in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Where indicated, COS1 cells were serum-starved for 24 h in DMEM containing 0.1% FBS, plus antibiotics. COS1 cells were transfected by the calcium phosphate precipitation method as described previously (12). Unless otherwise indicated were transfected using TCPTP pMT2 plasmid DNA at 20 μg/10-cm dish. Cells were washed three times with phosphate buffered saline (PBS) at 5–6 h after transfection and supplemented with fresh DMEM containing 10% FBS. Where indicated approximately 1–2 × 10^5 COS1 cells were electroporated in 250 μl of medium with 20 μg of TCPTP pMT2 plasmid at 200 V and 950 microfarads in 0.4-cm cuvettes and seeded into a 10-cm dish. Transfected or electroporated cells were collected at 36–48 h after transfection, or washed once with PBS at 24 h after transfection, supplemented with DMEM containing 0.1% FBS and processed at 48 h after transfection. The efficiency of electroporation, as assessed by 5-bromo-4-chloro-3-indolyl β-galactosidase staining of pCMV-β-galactosidase-electroporated COS1 cells, was routinely 50–75%.

**Immune Complex Kinase Reactions**

ERK2 and JNK Assays—COS1 cells, in 10-cm dishes, were transfected with 2 μg of HA-tagged ERK2 pJ3H plasmid or 5 μg of FLAG-tagged JNK pCMV plasmid and either 15 μg of pMT2 plasmid or 15 μg of the TC45 or TC45-D182A pMT2 plasmids. At 24 h after transfection, cells were washed once with PBS and serum-starved overnight for 24 h. Cells were then left unstimulated or stimulated with 100 ng/ml EGF for 15 min and processed for either ERK2 kinase assays as described previously (12) or JNK assays. For JNK assays, cells were lysed in 250 μl of immunoprecipitation (IP) lysis buffer (50 mM Tris, pH 7.5, 1 m M NaCl, 0.5 mM EDTA, and 1% Triton X-100) and the phospholipids were extracted and separated by thin layer chromatography on silica plates coated with potassium oxalate. v32P]Phosphatidylinositol was quantitated on a PhosphorImager using ImageQuant software (Molecular Dynamics).

**PKB/Akt Assays**—COS1 cells, in 10-cm dishes, were transfected with 5 μg of HA-tagged PKB/Akt pECE plasmid or 5 μg of pMT2 plasmid (containing 0.1% FBS, plus antibiotics) and stimulated with EGF (100 ng/ml) for 15 min. Cells were then washed 0.9 ml of IP lysis buffer, the lysates centrifuged (12,000 × g for 10 min at 4 °C) and HA-PKB/Akt immunoprecipitated from the supernatant with anti-HA antibody (12C10). The HA-PKB/Akt antibody accumulates at the cell periphery and colocalizes with the EGF receptor (12). At early time points after EGF stimulation, the TC45-D182A mutant accumulates at the cell periphery and colocalizes with the EGF receptor (11). At early time points after EGF stimulation, the TC45-D182A mutant accumulates at the cell periphery and colocalizes with the EGF receptor (11). To examine the role of TC45 in EGF receptor signaling, we have

**RESULTS**

TCPTP Regulates EGF Receptor-mediated PI 3-Kinase Signaling

**Cell Stimulation with Fibronectin**

COS1 cells were electroporated with pMT2 vector control, TC45, or TC45-D182A expression plasmids as indicated above. At 24 h after electroporation, cells were washed once with PBS at 24 h, washed twice with serum-free media and then harvested by limited trypsin-EDTA treatment (1 ml of 0.05% trypsin plus 5 μg EDTA in DMEM minus phenol red/10-cm dish of cells). After trypsin inhibition by soybean trypsin inhibitor (1 mg of chromatographically purified type I-S trypsin inhibitor (Sigma/10-cm dish of cells) in DMEM minus phenol red containing 0.25% (w/v) bovine serum albumin (BSA) radioimmunoassay grade, fraction V from Sigma), the cells were pelleted by centrifugation and washed twice with DMEM minus phenol red containing 0.25% (w/v) BSA and resuspended in DMEM minus phenol red containing 0.1% (w/v) BSA. The cells were held in suspension at 37 °C for 30 min prior to attachment for 1 h onto tissue culture dishes precoated with fibronectin (5 ml of 10 μg/ml human plasma fibronectin/10-cm dish incubated overnight at 4 °C and then resuspended with DMEM minus phenol red containing 0.25% (w/v) BSA and resuspended in DMEM minus phenol red containing 0.1% (w/v) BSA) at 37 °C for 30 min). Attached cells were rinsed twice in DMEM minus phenol red containing 0.1% (w/v) BSA, once in ice-cold PBS, and then collected in hot 3× Laemmli sample buffer containing 6% (w/v) β-mercaptoethanol. Proteins were resolved by SDS-PAGE and immunoblotted as indicated.

**Selectivity of TC45 on EGF Receptor-mediated PI 3-Kinase Signaling**

In response to stimulation with EGF, the TC45-D182A substrate trapping mutant forms a stable complex with the EGF receptor (12). At early time points after EGF stimulation, the TC45-D182A mutant accumulates at the cell periphery and colocalizes with the EGF receptor (11). Consistent with recognition of the tyrosine-phosphorylated EGF receptor at the plasma membrane. To examine the role of TC45 in EGF receptor signaling, we have
overexpressed wild type TC45 and the TC45-D182A mutant in COS1 cells and measured their effects on EGF-dependent activation of the MAPKs, extracellular signal-regulated kinase 2 (ERK2) and c-Jun N-terminal kinase (JNK) (Fig. 2). Consistent with our previous studies, we observed no apparent effect of TC45 on the activation of HA-tagged ERK2 (12). However, coexpression of TC45 inhibited the activation of FLAG-tagged JNK by 38.3 ± 7.8%. These results illustrate the ability of TC45 to regulate selectively signaling events emanating from the EGF receptor. Recent studies have indicated that EGF-induced activation of JNK, but not ERK2, is mediated by PI 3-kinase (16, 17). Consistent with these studies, we found that the PI 3-kinase inhibitor wortmannin had no effect on EGF-induced activation of ERK2 (Fig. 2, panel A, inset), but inhibited the activation of JNK by 57.1 ± 9.4% (Fig. 2, panel B). These results suggest that PI 3-kinase may be a target by which TC45 mediates its effects on the activation of JNK.

**TC45 Inhibits the EGF-Induced Activation of the Protein Kinase PKB/Akt**—Although the mechanism by which PI 3-kinase regulates the activation of JNK is not known, the ability of PI 3-kinase to regulate the activation of the protein kinase PKB/Akt, which mediates the effects of PI 3-kinase on cell proliferation and survival, is well documented (18–24). The lipid product of PI 3-kinase, phosphatidylinositol-3,4,5-triphosphate, binds to the pleckstrin homology domain of PKB/Akt and recruits it to the plasma membrane, where it is further activated by phosphorylation of Thr-308 and Ser-473. To determine whether TC45 regulates PI 3-kinase-mediated signaling processes, we examined the effects of TC45 and TC45-D182A on the EGF-induced stimulation of PKB/Akt, by coexpressing HA-tagged PKB/Akt with either TC45 or TC45-D182A in COS1 cells. The activity of HA-immunoprecipitated PKB/Akt was measured in vitro using the specific peptide substrate RPRAATF (15). Both wild type TC45 and the TC45-D182A mutant inhibited the EGF-induced activation of PKB/Akt by approximately 60–70% (Fig. 3). Importantly, under similar conditions, expression of the ER-localized spliced variant TC48 had no apparent effect (Fig. 3, panel B). These results demonstrate that simple overexpression of a PTP is not sufficient to inhibit PKB/Akt activation and underscores the importance of proper subcellular localization for the control of TCPTP action.

**TC45 Inhibits EGF-induced PI 3-Kinase Activity**—As PKB/Akt is not tyrosine-phosphorylated it cannot serve as a substrate of TC45. Consequently, it is likely that TC45 exerts its effects on PKB/Akt, and possibly JNK, at a step upstream in the signaling pathway by regulating negatively the EGF-induced activation of PI-3 kinase. PI 3-kinase is activated by associating with sites of tyrosine phosphorylation generated by activated receptor tyrosine kinases (25–29). Therefore, PI 3-kinase activity can be measured in anti-phosphotyrosine immunoprecipitates to determine the extent by which it is stimulated in response to EGF (29). To examine the effects of TC45 on PI 3-kinase activity, we electroporated COS1 cells with expression plasmids for either vector control, TC45, or TC45-D182A and measured PI 3-kinase activity in anti-phosphotyrosine immunoprecipitates. Under these conditions TC45 and TC45-D182A inhibited the EGF-induced PI 3-kinase activity by ~50% (Fig. 4, panel A). Furthermore, we observed that the phosphorylation of PKB/Akt on serine 473 was inhibited by a similar extent, as measured in lysates of these electroporated cells (Fig. 4, panel B). Since the electroporation efficiency in these experiments was also in the order of 50–75% (data not shown), these results suggest that overexpression of TC45 can inhibit almost completely the activation of PI 3-kinase and PKB/Akt. These results are consistent with the ~70% inhibition of the EGF-induced activity of HA-tagged PKB/Akt (Fig. 3).

**TC45 Acts Upstream of PI 3-Kinase and Inhibits the Recruitment of PI 3-Kinase to the EGF Receptor**—To examine whether TC45 can inhibit the activation PKB/Akt independently of its effects on PI 3-kinase, we cotransfected COS1 cells with expression plasmids encoding HA-tagged PKB/Akt and a constitutively active form of the PI 3-kinase catalytic subunit, p110K227E (30), together with either vector control, TC45 or TC45-D182A and measured the effects on HA-PKB/Akt activity. Expression of TC45 and TC45-D182A did not inhibit the p110K227E-induced activation of PKB/Akt either in the absence or presence of EGF (Fig. 5). Therefore, these data illustrate that TC45 must act to suppress the activation of PI...
3-kinase, thereby inhibiting the downstream activation of PKB/Akt. These observations raise the issue of how TC45 suppresses the activation of PI 3-kinase. One possibility is that TC45 acts on the EGF receptor, to inhibit the recruitment of the PI 3-kinase p85 regulatory subunit, thereby preventing PI 3-kinase activation and the subsequent activation of PKB/Akt. Whereas the wild type TC45 has the capacity to dephosphorylate the EGF receptor, the TC45-D182A substrate trapping mutant would bind to phosphotyrosine sites on the EGF receptor, thus competing with SH2 domain-containing signaling molecules and thereby interfering with concomitant PI 3-kinase activation. We investigated whether expression of TC45 could inhibit the recruitment of the p85 PI 3-kinase subunit to the EGF receptor (Fig. 6). First, we examined the ability of TC45 to reduce the amount of p85 in anti-phosphotyrosine antibody immunoprecipitates from EGF-stimulated COS1 cells that had been electroporated with vector control, TC45, or TC45-D182A expression plasmids. Following EGF stimulation of control cells, the p85 regulatory subunit of PI 3-kinase could be detected in anti-phosphotyrosine immunoprecipitates. However, tyrosine-phosphorylated p85 was not detectable in cell lysates or anti-phosphotyrosine immunoprecipitates from EGF-stimulated cells (data not shown), suggesting that the p85 in anti-phosphotyrosine immunoprecipitates was associated with other phosphotyrosine-containing proteins. We found that wild type and mutant TC45 significantly decreased the amount of p85 in anti-phosphotyrosine immunoprecipitates (Fig. 6, panel A) and the extent of this inhibition was similar to the observed decrease in PI 3-kinase activity in anti-phosphotyrosine antibody immunoprecipitates (Fig. 4, panel A). Since one
of the major phosphotyrosine-containing protein in anti-phosphotyrosine antibody immunoprecipitates from EGFS
stimulated COS1 cells is the EGF receptor (12), the data infer that TC45 inhibits the recruitment of p85 to the EGF receptor. This was confirmed directly by immunoprecipitating the EGF receptor from COS1 cells that had been electroporated with either vector control, TC45 or TC45-D182A expression plasmids. Expression of either TC45 or TC45-D182A inhibited the EGF-induced association of the p85 regulatory subunit with the EGF receptor (Fig. 6, panel B).

TC45 Inhibits the Integrin-induced and EGF Receptor-mediated Activation of PKB/Akt but Not ERK2—Moro et al. (31) have shown recently that in human primary skin fibroblasts and in ECV304 endothelial cells, integrins can utilize the EGF receptor to transduce extracellular matrix-induced signaling pathways. This integrin-mediated activation of the EGF receptor is independent of EGF. In the present study, we have demonstrated that COS1 cells can activate the EGF receptor, PKB/Akt and ERK2 when plated on fibronectin (Fig. 7). The activation of PKB/Akt and ERK2 can be inhibited by tyrphostin AG1478 (Fig. 7), which is a specific inhibitor of the EGFR receptor (32), suggesting that the activation of PKB/Akt and ERK2 following integrin ligation is mediated by the EGF receptor.

Moreover, as in the case of EGF-mediated signaling, activation of PKB/Akt, but not ERK2, is PI 3-kinase-dependent and is inhibited by wortmannin (Fig. 7). We examined whether TC45 could inhibit the EGF receptor-mediated activation of PKB/Akt following integrin ligation. COS1 cells electroporated with vector control, TC45, or TC45-D182A expression plasmids were serum-starved and then detached and replated onto fibronectin-coated dishes. First, we examined whether the EGF receptor could serve as a TC45 substrate following cell attachment, by comparing the state of tyrosine phosphorylation of the EGF receptor in cell lysates before and after plating on fibronectin. Compared with vector control, the EGF receptor was dephosphorylated by wild type TC45 but protected from dephosphorylation by the TC45-D182A substrate trapping mutant (Fig. 8, panel A). These results are consistent with the EGF receptor being a direct substrate of TC45, as we have previously demonstrated for EGF-induced activation (12). We next assessed the effect of overexpressing TC45 or TC45-D182A on the extracellular matrix-induced activation of PKB/Akt, by immunoblot analysis of the cell lysates using antibodies specific for PKB/Akt phosphorylated on Ser-473 (phospho-Akt). Phospho-Akt immunoblots were stripped and reprobed with polyclonal antibodies specific for PKB/Akt.

FIG. 4. TC45 inhibits the EGF-induced and phosphotyrosine-associated PI 3-kinase activity. COS1 cells were electroporated with either pMT2 vector control or plasmids expressing TC45 or TC45-D182A (TC45D). Electroporated cells were serum-starved and then stimulated with EGF (100 ng/ml) for 15 min. A, Cells were lysed and the phosphotyrosine-containing proteins were immunoprecipitated and assayed for PI 3-kinase activity. Labeled lipids were resolved by thin layer chromatography, and [32P]phosphatidylinositol was quantitated on a PhosphorImager. Negligible [32P]phosphatidylinositol was detected in immunoprecipitates from serum-starved cells not stimulated with EGF. Results from four independent experiments (mean ± standard errors) were expressed as percentage of activity of vector control after EGF stimulation. B, lysates from electroporated cells were resolved by SDS-PAGE and immunoblotted with monoclonal antibodies specific for PKB/Akt phosphorylated on Ser-473 (phospho-Akt). Phospho-Akt immunoblots were stripped and reprobed with monoclonal antibodies specific for PKB/Akt.

FIG. 5. TC45 does not inhibit the activation of PKB/Akt mediated by a constitutively active PI 3-kinase. COS1 cells were co-electroporated with constitutively active p110K227E, HA-tagged PKB/Akt and vector control, TC45, or TC45-D182A. Transfected cells were serum-starved and either left unstimulated or stimulated with EGF (100 ng/ml) for 15 min. Cells were then lysed, and HA-tagged PKB/Akt was precipitated and assayed using peptide substrate. Activity was normalized for HA-tagged PKB/Akt protein.

DISCUSSION

Activation of the EGF receptor PTK by ligand results in receptor dimerization and autophosphorylation on tyrosyl residues. The phosphotyrosyl residues that are produced serve as docking sites for SH2 domain-containing signaling molecules, leading to the assembly of multiprotein signaling complexes required for cell growth, proliferation, and survival. Although significant progress has been made in defining the tyrosine phosphorylation-dependent signaling pathways downstream of the EGF receptor, relatively little is known about which members of the PTP family serve to antagonize these signaling events. Through the use of substrate trapping mutants, we have demonstrated previously that the nuclear, 45-kDa form of TCPTP, TC45, can exit the nucleus in response to EGF and...
TCPTP regulates EGF receptor-mediated PI 3-kinase signaling

In this study, we have characterized the ability of TC45 to regulate EGF receptor-induced signaling. TC45 inhibited the EGF receptor-mediated activation of PI 3-kinase and PKB/Akt. Thus, TC45 can regulate selectively Shc-dependent signaling events.

In this study, we have characterized the ability of TC45 to regulate EGF receptor-induced signaling. TC45 inhibited the EGF receptor-mediated activation of PI 3-kinase and PKB/Akt. Thus, TC45 can regulate selectively Shc-dependent signaling events.

Binding of p85 via its SH2 domain to tyrosine-phosphorylated receptors allows for the recruitment and activation of the PI 3-kinase p110 catalytic subunit. The five major autophosphorylation sites on the EGF receptor do not fit the Tyr-X-X-Met motif, but under certain circumstances Src can phosphorylate the EGF receptor on Tyr\(^{p325}\) which has the motif for p85 binding (34). Also, others have reported that the SH2 domains of p85 can interact directly with the tyrosine-phosphorylated EGF receptor (35). Alternatively, the EGF receptor can phosphorylate docking proteins such as p120\(^{cbl}\) and Gab1 (36, 37), which in turn bind p85 and therefore recruit PI 3-kinase activity. Regardless of the precise mechanism by which PI 3-kinase associates with the EGF receptor, tyrosine phosphorylation of the EGF receptor is necessary for this event. By dephosphorylating the EGF receptor, TC45 can inhibit the association of p85 and concomitant activation of PI 3-kinase and PKB/Akt.

The inhibition of p85 recruitment to the EGF receptor that we observed is consistent with the effects of TC45 and TC45-D182A on the activities of PI 3-kinase and PKB/Akt but not entirely consistent with their effects on JNK. Although both TC45 and TC45-D182A could equally inhibit the recruitment of p85 and the activation of PI 3-kinase and PKB/Akt, we observed that only wild type TC45 inhibited JNK. However, it is important to note that, unlike PKB/Akt, whose EGF-induced activation can be completely inhibited by the PI 3-kinase inhibitor wortmannin, JNK activity is only partially inhibited by this PI 3-kinase antagonist (this study and Ref. 16). In addition, dominant negative forms of the p85 regulatory subunit only partially inhibit the EGF-induced activation of JNK.

Therefore, other signaling events in addition to PI 3-kinase would seem to be necessary for EGF-mediated activation of JNK. Until the exact nature of the PI 3-kinase-mediated activation of JNK has been defined, it will be difficult to speculate as to the mechanism of differential regulation of JNK and PKB/Akt by TCPTP.
mediated activation of PKB/Akt but not ERK2. The prestained 205-kDa molecular size standard (Bio-Rad) is indicated in anti-pTyr and EGF receptor blots.

Moro et al. (31) have recently reported that adhesion of human primary skin fibroblasts or ECV304 endothelial cells to fibronectin results in EGF receptor activation in the absence of EGF and that this is necessary for the integrin-mediated activation of the MAPK ERK1. We found that, in COS1 fibroblast cells, the integrin-mediated activation of ERK2 as well as PKB/Akt, and TCPTP and (B) phospho-Akt, Akt, or ERK2. The prestained 205-kDa molecular size standard (Bio-Rad) is indicated in anti-pTyr and EGF receptor blots.

of EGF receptor signaling to MAPK (46–51). However, to our knowledge, our data represent the first occasion on which a PTP has been shown to act upstream of PI 3-kinase, most likely on the EGF receptor, to regulate negatively EGF receptor-mediated and PI 3-kinase-dependent signaling events. Thus, TC45 may serve as an important target for intervention in tumors where excessive EGF receptor-mediated PI 3-kinase signaling contributes to the disease.

Acknowledgments—We thank David Hill for the TCPTP CF4 antibody and Mike Weber for the ERK2 antibody. We also thank Jörg Heierhorst for critical reading of the manuscript and Richard Pearson for help with the PKB/Akt assays.

REFERENCES

1. Tonks, N. K., and Neel, B. G. (1996) Cell 87, 365–368
2. Tonks, N. K. (1996) Adv. Pharmacol. 36, 91–119
3. Neel, B. G., and Tonks, N. K. (1997) Curr. Opin. Cell Biol. 9, 193–204
4. Cool, D. E., Tonks, N. K., Charbonneau, H., Fischer, E. H., and Krebs, R. G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7280–7284
5. Lorenzen, J. A., Dadabay, C. Y., and E. H., F. (1995) J. Cell Biol. 131, 631–643
6. Mostinger, B., Jr., Tillmann, U., Westphal, H., and Tremblay, M. L. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 499–503
7. Champion-Arnaud, P., Gesnel, M. C., Foulkes, N., Roncin, C., Sassone-Corsi, P., and Breathnach, R. (1991) Oncogene 6, 1203–1209
8. Tillmann, U., Wagner, J., Boerboom, D., Westphal, H., and Tremblay, M. L. (1994) Mol. Cell. Biol. 14, 3030–3040
9. Tiganis, T., Flint, A. J., Adam, S. A., and Tonks, N. K. (1997) J. Biol. Chem. 272, 21545–21557
10. Garton, A. J., Flint, A. J., and Tonks, N. K. (1996) Mol. Cell. Biol. 16, 6408–6418
11. Flint, A. J., Tiganis, T., Barford, D., and Tenks, N. K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1680–1685
12. Tiganis, T., Bennett, A. M., Ravichandran, K. S., and Tonks, N. K. (1998) Mol. Cell. Biol. 18, 1622–1634
13. Joneson, T., McDonough, M., Bar-Sagi, D., and Van Aelst, A. (1996) Science 274, 1374–1376
14. Shaw, L. M., Rabinovitz, I., Wang, H. H., Toker, A., and Mercurio, A. M. (1997) Cell 91, 949–960
15. Alesci, D. R., Caudwell, F. B., Andjelkovic, M., Hemmings, B. A., and Cohen, P. (1996) FEBS Lett. 399, 333–338
16. Logan, S. K., Falasca, M., Hu, P., and Schlessinger, J. (1997) Mol. Cell. Biol. 17, 5784–5790
17. King, W. G., Mattaliano, M. D., Chan, T. O., Tsichlis, P. N., and Brugge, J. S. (1995) Mol. Cell. Biol. 15, 14046–1418
18. Franke, T. F., Kaplan, D. R., Cantley, L. C., and Toker, A. (1997) Science 273, 665–668
19. Franke, T. F., Kaplan, D. R., and Cantley, L. C. (1997) Cell 88, 435–437
20. Boudewijn, M., Burgering, T., and Coffer, P. J. (1995) Nature 376, 599–602
21. Downward, J. (1998) Science 279, 673–674
22. Wong, D., Stephens, L. R., Copeland, T., Gaffney, P. R., Reese, C. B., Painter, G. F., Holmes, A. B., McCormick, F., and Hawkins, P. T. (1997) Science 277, 567–570
23. Hemmings, B. A. (1997) Science 275, 628–630
24. Anderson, K. E., Caudwell, J., Stephens, L. R., and Hawkins, P. T. (1998) Curr. Biol. 8, 684–691
25. Backer, J. M., Myers, M. G., Jr., Shoelson, S. E., Chin, D. J., Sun, X. J., Miralpeix, M., Hu, P., Maragos, R., Skeikin, E. Y., Schlessinger, J., and White, M. P. (1992) EMBO J. 11, 3469–3479
26. Yu, J., Zhang, Y., McIlroy, J., Rordorf-Nikolic, T., Orr, G. A., and Backer, J. M. (1998) Mol. Cell. Biol. 18, 1379–1387
27. Yu, J., Wijasus, C., and Backer, J. M. (1998) J. Biol. Chem. 273, 30199–30203
28. Carpenter, C. L., Auger, K. R., Chanudhuri, M., Yoakim, M., Schaffhausen, B., Shoelson, S., and Cantley, L. C. (1999) J. Biol. Chem. 274, 9497–9483
29. Hu, P., Maragos, R., Skolnik, E. Y., Lamers, R., Ulrich, A., and Schlessinger, J. (1999) Mol. Cell. Biol. 12, 981–990
30. Kauffmann-Zeh, A., Rodriguez-Viciana, P., Ulrich, E., Gilbert, C., Coffer, P., and Downward, J. (1997) Nature 385, 544–548
31. Moro, L., Venturino, M., Bozzo, C., Silengo, L., Altuda, F., Benuinot, L., Tarone, G., and Dellipinto, P. (1998) EMBO J. 17, 6622–6632
32. Levitzki, A., and Gazit, A. (1985) Science 227, 1782–1788
33. Songyang, Z., Shoelson, S. E., Chaudhuri, M., Gish, G., Toker, A., Warner, W. G., K救援, B. L., Henkes, F., and Hawkins, P. T. (1997) J. Biol. Chem. 272, 767–778
34. Stover, D. R., Becker, M., Lietzanz, J., and Lydon, N. B. (1995) J. Biol. Chem. 270, 15591–15597
35. Moscatello, D. K., Holgado-Madurga, M., Emlet, D. R., Montgomery, R. B., and Wong, A. J. (1998) J. Biol. Chem. 273, 290–296
36. Meisner, H., Conway, B. R., Hartley, D., and Czech, M. P. (1995) Mol. Cell. Biol. 15, 3571–3578
37. Holgado-Madurga, M., Emlet, D. R., Moscatello, D. K., Godwin, A. K., and Wong, A. J. (1996) Nature 379, 560–564
38. Schlegel, J., Merdes, A., Stumm, G., Albert, P. F., Forsting, M., Hynes, N., and Kessler, M. (1994) J. Biol. Chem. 269, 72–77
39. Garcia de Palacio, I. E., Adams, G. P., Staudenrath, P., Wong, A. J., Testa, J. R., Bigner, D. D., and Wein, I. M. (1993) Cancer Res. 53, 3217–3220
40. Harris, A. L., Nicholson, S., Sainsbury, R., Wright, C., and Farnoud, J. (1992) J. Natl. Cancer Inst. Monogr. 11, 181–187

FIG. 8. TC45 inhibits the integrin-induced and EGF receptor-mediated activation of PKB/Akt but not ERK2. COS1 cells were electroporated with pMT2 vector control, TC45 or TC45-D182A (TC45D), serum-starved, detached, and kept in suspension (Sus) for 30 min before replating onto fibronectin (Fib) for 1 h. Cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies specific for (A) phosphotyrosine (pTyr), the EGF receptor (EGFR), and TCPTP and (B) phospho-Akt, Akt, or ERK2. The prestained 205-kDa molecular size standard (Bio-Rad) is indicated in anti-pTyr and EGF receptor blots.
41. Wong, A. J., Ruppert, J. M., Bigner, S. H., Grzeschik, C. H., Humphrey, P. A., Bigner, D. S., and Vogelstein, B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 2965–2969
42. Wong, A. J., Bigner, S. H., Bigner, D. D., Kinzler, K. W., Hamilton, S. R., and Vogelstein, B. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6899–6903
43. Hunts, J., Ueda, M., Ozawa, S., Abe, O., Pastan, I., and Shimizu, N. (1985) Jpn. J. Cancer Res. 76, 663–666
44. Libermann, T. A., Nusbaum, H. R., Razon, N., Kris, R., Lax, I., Soreq, H., Whittle, N., Waterfield, M. D., Ullrich, A., and Schlessinger, J. (1985) Nature 313, 144–147
45. Di Fiore, P. P., Pierce, J. H., Fleming, T. P., Hazan, R., Ullrich, A., King, C. R., Schlessinger, J., and Aaronson, S. A. (1987) Cell 51, 1063–1070
46. Bennett, A. M., Hausdorff, S. F., Oreilly, A. M., Freeman, R. M., and Neel, B. G. (1996) Mol. Cell. Biol. 16, 1189–1202
47. Su, L., Zhao, Z., Bouchard, P., Banzville, D., Fischer, E. H., Krebs, E. G., and Shen, S.-H. (1996) J. Biol. Chem. 271, 10395–10390
48. You, M., and Zhao, Z. (1997) J. Biol. Chem. 272, 23376–23381
49. Keilhack, H., Tenev, T., Nyakatura, E., Godovac-Zimmermann, J., Nielsen, L., Seedorf, K., and Bohmer, F.-D. (1998) J. Biol. Chem. 273, 24839–24846
50. Tomic, S., Greiser, U., Lammers, R., Kharitonenkov, A., Imanitov, E., Ullrich, A., and Bohmer, F.-D. (1998) J. Biol. Chem. 273, 21277–21284
51. Deb, T. B., Wong, L., Salomon, D. S., Zhou, G., Dixon, J. E., Gutkind, J. S., Thompson, S. A., and Johnson, G. R. (1998) J. Biol. Chem. 273, 16643–16646