Hepatocyte Growth Factor Induces ERK-dependent Paxillin Phosphorylation and Regulates Paxillin-Focal Adhesion Kinase Association*

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Hepatocyte growth factor (HGF) modulates cell adhesion, migration, and branching morphogenesis in cultured epithelial cells, events that require regulation of cell-matrix interactions. Using mIMCD-3 epithelial cells, we studied the effect of HGF on the focal adhesion proteins, focal adhesion kinase (FAK) and paxillin and their association. HGF was found to increase the tyrosine phosphorylation of paxillin and to a lesser degree FAK. In addition, HGF induced association of paxillin and activated ERK, correlating with a gel retardation of paxillin that was prevented with the ERK inhibitor U0126. The ability of activated ERK to phosphorylate and induce gel retardation of paxillin was confirmed \textit{in vitro} in both full-length and amino-terminal paxillin. Several potential ERK phosphorylation sites in paxillin flank the paxillin-FAK association domains, so the ability of HGF to regulate paxillin-FAK association domains was examined. HGF induced an increase in paxillin-FAK association that was inhibited by pretreatment with U0126 and reproduced \textit{in vitro} phosphorylation of paxillin with ERK. The prevention of the FAK-paxillin association with U0126 correlated with an inhibition of the HGF-mediated FAK tyrosine phosphorylation and inhibition of HGF-dependent cell spreading and adhesion. An examination of cellular localization of FAK and paxillin demonstrated that HGF caused a condensation of focal adhesion complexes at the leading edges of cell processes and FAK-paxillin co-localization in these large complexes. Thus, these data suggest that HGF can induce serine/threonine phosphorylation of paxillin most probably mediated directly by ERK, resulting in the recruitment and activation of FAK and subsequent enhancement of cell spreading and adhesion.

Hepatocyte growth factor (HGF) is a heparin-binding protein that is produced primarily by fibroblasts and peritubular mesenchymal cells (1), and that can stimulate the c-Met receptor to initiate at least three distinct responses, mitogenesis, scattering/migration, and branching tubule formation (3, 4). The ability of HGF to induce cell migration and morphogenesis implies that it must initiate intracellular signaling events that regulate cell-matrix interactions. In support of this finding, HGF has recently been shown to directly stimulate integrin-dependent tumor cell adhesion to laminin, fibronectin, and vitronectin (5–7). In studies using immortalized renal murine inner medullary collecting duct (mIMCD-3) epithelial cells, we have demonstrated that HGF induces increased adhesion to fibronectin and type 1 collagen, and that this increase in adhesion is dependent on ERK activation (8).

Presently, there is little known about the mechanism for the HGF-dependent increase in epithelial cell adhesion. In two studies (5, 6), the stimulation with HGF has been found to cause tyrosine phosphorylation of FAK and to mediate an increased linkage between the integrin complexes and the actin cytoskeleton. In breast carcinoma cells, Beviglia and Kramer (6) found that HGF induced tyrosine phosphorylation of FAK in adherent cells but failed to induce FAK activation in suspended cells. Similarly, Chen \textit{et al.} (9) found that stimulation of A293 cells with HGF resulted in FAK phosphorylation on tyrosine. This phosphorylation was most pronounced in cells adherent to plastic dishes or polylysine-coated dishes. Interestingly, A293 cells adherent to dishes coated with fibronectin revealed a marked increase in basal tyrosine phosphorylation of FAK with only a modest further increase following the activation of c-Met.

In this study, we examined the effects of c-Met activation on the regulation of FAK and paxillin in renal tubular epithelial cells. We found that in mIMCD-3 cells, FAK exhibits a high basal level of tyrosine phosphorylation with only a modest increase following HGF stimulation. In contrast, paxillin demonstrated a marked increase in tyrosine phosphorylation following HGF stimulation as well as a decrease in gel mobility reminiscent of serine/threonine phosphorylation. This gel retardation was found to be dependent on HGF-mediated ERK activation and to correlate with an association of paxillin and activated ERK. We demonstrate that ERK can phosphorylate paxillin \textit{in vitro}, resulting in an increase in the association of paxillin and FAK. Finally, HGF was found to induce paxillin-FAK association in adherent cells in an ERK-dependent manner.

EXPERIMENTAL PROCEDURES

Cells and Reagents—Experiments were performed with immortalized mIMCD-3 cells of ureteric bud origin (10) that are known to express the c-Met receptor and undergo striking tubulogenesis in response to HGF (4). All cells were grown in Dulbecco’s modified Eagle’s/F12 medium supplemented with 10% fetal calf serum. Antibody sources for immunoprecipitation, Western blotting, and immunofluorescence are paxillin monoclonal antibody (Transduction Laboratories), FAK monoclonal and polyclonal antibodies (Santa Cruz Biotechnology), and phos-

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FIG. 1. HGF induces tyrosine phosphorylation of paxillin and FAK. A, mIMCD-3 cells were plated on 4 h (left panel) or 36 h (right panel) prior to stimulation with HGF and immunoprecipitation with anti-FAK. The immunoprecipitates were then blotted with anti-pY (upper panel) or anti-FAK (lower panel). HGF induces a modest increase in tyrosine phosphorylation of FAK in newly plated mIMCD-3 cells but not in cells plated for a prolonged time. B, mIMCD-3 cells were plated for 36 h followed by stimulation with HGF and immunoprecipitation with anti-paxillin, which was followed by immunoblotting with anti-pY (upper panel) or anti-paxillin (lower panel). HGF induced a 2.6-fold increase in tyrosine phosphorylation of paxillin (-fold increase based on n = 4, range 1.6–3.7).

FIG. 2. HGF induces a MAPK-dependent gel retardation of paxillin. A, mIMCD-3 cells were stimulated with HGF (40 ng/ml) for 10 or 30 min ± pretreatment with U0126 (10 μM) for 30 min. Twenty micrograms of whole cell lysates were separated by prolonged SDS-PAGE and immunoblotted with anti-paxillin (upper panel), anti-phospho-ERK (middle panel), and anti-total ERK (lower panel). HGF stimulation caused gel retardation of paxillin that was prevented by 10 μM U0126, correlating with inhibition of ERK activation. B, mIMCD-3 cells were stimulated with HGF ± U0126, and anti-paxillin immunoprecipitation was performed followed by immunoblotting with anti-pY (upper panel) or anti-paxillin (lower panel). U0126 did not inhibit HGF-dependent tyrosine phosphorylation of paxillin.
FIG. 3. Association and phosphorylation of paxillin by ERK. A, mIMCD-3 cells were stimulated with HGF for 10 min, and cell lysates were then incubated with either anti-paxillin antibody (right panel) or recombinant amino-terminal paxillin (gst-N-paxillin or GST control beads, left panel). Precipitates were washed, separated by SDSPAGE, and immunoblotted with an anti-phospho-MAPK antibody that recognizes activated ERK1 and ERK2 (upper panel) and anti-paxillin (lower panel). Stimulation with HGF results in the association of paxillin and activated ERK2. B, mIMCD-3 cells were treated with U0126 followed by immunoprecipitation of paxillin. The paxillin was then phosphorylated in vitro with purified active ERK and [32P]ATP, isolated using SDS-PAGE, and detected by autoradiography (upper panel) and anti-paxillin immunoblot (lower panel). ERK phosphorylates paxillin immunoprecipitated from control cells but only weakly phosphorylates paxillin from HGF-stimulated cells. The inhibition of ERK activation with U0126 prior to HGF stimulation restores the ability of ERK to phosphorylate paxillin in vitro. C, quantification of four experiments were performed as described in B. Autoradiograms were quantitated using the NIH Image software and normalized to the amount of paxillin immunoprecipitated for each condition. Results were plotted as the mean ± S.E. p < 0.01 for HGF versus control. D, autoradiogram (upper panel) and anti-paxillin immunoblot (lower panel) of an in vitro kinase assay of GST-NH2-terminal paxillin incubated with active ERK and [32P]ATP for the indicated times. ERK phosphorylates the amino terminus of paxillin in vitro resulting in a reduction of gel mobility, consistent with that seen following HGF stimulation in the intact cell (see Fig. 2A).

ng/ml) ± U0126 (10 μM). Cells were washed twice with phosphate-buffered saline and fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.5% Triton X-100 for 5 min, blocked with 3% bovine serum albumin for 1 h, and then processed for immunofluorescence. Coverslips were incubated sequentially for 1 h each at room temperature with monoclonal anti-FAK antibody (1:100, Transduction Laboratories) and rhodamine-conjugated goat anti-mouse IgG (1:64, Sigma) followed by fluorescein isothiocyanate-conjugated monoclonal anti-paxillin (1:100, Transduction Laboratories). Coverslips were then washed three times in phosphate-buffered saline with 0.5% Triton X-100, mounted on glass slides in Antifade-mounting medium (Molecular Probes), and visualized at ×40 using a Zeiss epifluorescence confocal microscope.

Cell Adhesion Assay—Cell adhesion was performed as described previously (8). mIMCD-3 cells were serum-starved for 24 h and harvested by washing in 1× EDTA for 10 min. The cells were counted and then treated with HGF (40 ng/ml) or vehicle control for 10 min prior to plating 2 × 104 cells/well on 96 well plates in Dulbecco’s modified Eagle’s/F12 medium for 30 min. In experiments using the MEK inhibitor U0126, cells were preincubated with U0126 (10 μM) or vehicle control for 10 min prior to stimulation with HGF. Non-adherent cells were removed by washing twice with phosphate-buffered saline, and the remaining cells were quantitated by the method of Oliver et al. (13). Cells were fixed for 30 min with 10% formalin, stained for 30 min with 1% methylene blue, and washed three times with 0.01 M boric acid, pH 8.5. The intracellular dye was extracted with 0.1 N HCl/ethanol (1:1) for 1 h at room temperature and quantified by absorbance at 655 nm. Photomicroscopy was performed using a Nikon microscope equipped with Hoffman modulation optics.

RESULTS AND DISCUSSION

HGF Induces Phosphorylation of the Focal Adhesion Proteins FAK and Paxillin—To examine the effect of c-Met activation on FAK phosphorylation in renal epithelial cells, anti-phosphotyrosine blotting of FAK immunoprecipitated from mIMCD-3 cells was performed. In contrast to the results reported in breast carcinoma cells (6), FAK is heavily tyrosine-phosphorylated at base line in stably adherent mIMCD-3 cells with little change following stimulation with HGF (Fig. 1A, right panel). Because matrix-dependent integrin activation has been found to increase FAK phosphorylation (9), we examined the ability of HGF to activate FAK in cells plated for only 4 h to limit endogenous matrix deposition. In these experiments, base-line FAK phosphorylation was diminished, and HGF did stimulate a modest increase in FAK phosphorylation (Fig. 1A, left panel).

We next examined the focal adhesion complex protein paxillin. Paxillin is a key scaffolding protein between the focal adhesion complex and the actin cytoskeleton that is known to bind to FAK and mediate FAK recruitment to focal adhesion complexes and that is tyrosine-phosphorylated by FAK and/or src in response to integrin engagement (14–17). In addition to cell adhesion, various extracellular stimuli including bombesin, platelet-derived growth factor, nerve growth factor, and angiotensin II have been shown to induce tyrosine phosphorylation of paxillin (18–21). In subconfluent mIMCD-3 cells, HGF induced a 2.6-fold increase in tyrosine phosphorylation of paxillin (Fig. 1B, fold increase based on n = 4, range 1.6–3.7). Of note, fully confluent cells demonstrated higher basal tyrosine phosphorylation of paxillin with a smaller increase in response to HGF stimulation (data not shown).
HGF-mediated stimulation of FAK phosphorylation is prevented by the treatment with HGF/H11006. Immunoblotting with anti-pY (\(\text{A}\)) result in association of paxillin and FAK. mIMCD-3 cells were stimulated with HGF, immunoprecipitated with anti-FAK, and immunoblotted with anti-FAK (upper panel) or anti-paxillin (lower panel). HGF stimulates an association of paxillin and FAK that is blocked by the MEK inhibitor U0126. A right panel, amino-terminal paxillin (gst-N-paxillin) was incubated with either inactive or active ERK for 30 min followed by incubation with lysates from unstimulated mIMCD-3 cells for 1 h. Precipitates were washed, separated by SDS-PAGE, and immunoblotted with anti-FAK (upper panel) and anti-paxillin (lower panel). In vitro phosphorylation of paxillin by active ERK markedly increases the phosphorylation of paxillin, because both the 68 and 70 kDa forms of paxillin are tyrosine-phosphorylated (Fig. 2B, lane 3), and the inhibition of ERK activation did not prevent tyrosine phosphorylation of paxillin (Fig. 2B, lane 4). These results suggest that HGF can induce both tyrosine and serine/threonine phosphorylation of paxillin, and that the latter occurs downstream of ERK1/ERK2 activation.

**FIG. 4.** **HGF induces an ERK-dependent association of paxillin and FAK.** A, left panel, mIMCD-3 cells were stimulated with HGF ± U0126, immunoprecipitated with anti-paxillin, and immunoblotted with anti-FAK (upper panel) or anti-paxillin (lower panel). HGF stimulates an association of paxillin and FAK that is blocked by the MEK inhibitor U0126. Right panel, amino-terminal paxillin (gst-N-paxillin) was incubated with either inactive or active ERK for 30 min followed by incubation with lysates from unstimulated mIMCD-3 cells for 1 h. Precipitates were washed, separated by SDS-PAGE, and immunoblotted with anti-FAK (upper panel) and anti-paxillin (lower panel). In vitro phosphorylation of paxillin by active ERK markedly increases the association of paxillin and FAK. B, mIMCD-3 cells were stimulated with HGF, immunoprecipitated with anti-FAK, and immunoblotted with paxillin (upper panel) or FAK (lower panel), recapitulating the result in A. C, mIMCD-3 cells were plated for 4 h followed by stimulation with HGF ± U0126, immunoprecipitation with anti-FAK, and immunoblotted with anti-pY (upper panel) or anti-FAK (lower panel). The HGF-mediated stimulation of FAK phosphorylation is prevented by the inhibition of MEK1 and MEK2.

In the course of these experiments, we noted that in unstimulated cells paxillin primarily runs as one band at a molecular mass of 68 kDa (Fig. 2A). Following treatment with HGF, the amount of paxillin at 68 kDa decreased, and a new band appeared at ~70 kDa, a reduction in gel mobility that is suggestive of proteins phosphorylated on serine-proline and/or threonine-proline motifs (22). Of note, this reduction of gel mobility was only detectable if the gels were run for prolonged periods until paxillin nearly exited the gel. Because we have previously found that HGF-dependent activation of the serine/threonine kinase ERK is critical for the phenotype of cell migration and adhesion (8, 23), we examined the possibility that the gel retardation of paxillin was attributed to HGF-stimulated ERK activation by using the selective MEK inhibitor U0126. This compound has been shown to inhibit MEK1, MEK2 (24), and MEK5 (23) at concentrations of <50 \(\mu M\) but does not inhibit MEK3, MEK4, MEK6, protein kinase \(\text{Ca}\), protein kinase A, PDK1, or other tested serine/threonine kinases (24). We have previously found that at concentrations of 10–20 \(\mu M\), U0126 does not alter HGF-mediated c-Met or Gab1 tyrosine-phosphorylation or phosphatidylinositol 3-kinase activation (25). The pretreatment of mIMCD-3 cells with U0126 completely prevented HGF-induced ERK1/ERK2 phosphorylation (Fig. 2A, middle panel) and eliminated the HGF-induced paxillin gel shift (Fig. 2A, upper panel). Of note, ERK activation does not appear to play a role in the HGF-mediated tyrosine phosphorylation of paxillin, because both the 68 and 70 kDa forms of paxillin are tyrosine-phosphorylated (Fig. 2B, lane 3), and the inhibition of ERK activation did not prevent tyrosine phosphorylation of paxillin (Fig. 2B, lane 4). These results suggest that HGF can induce both tyrosine and serine/threonine phosphorylation of paxillin, and that the latter occurs downstream of ERK1/ERK2 activation.

**FIG. 5.** **HGF causes a condensation of focal adhesions with enhanced FAK-paxillin co-localization.** mIMCD-3 cells were plated on glass coverslips and treated ± HGF ± U0126 followed by fixation and immunostaining for paxillin (fluorescein isothiocyanate-conjugated, green) and FAK (tetramethylrhodamine isothiocyanate-labeled secondary antibody, red). Areas of co-localization were detected as yellow. Confocal images at the cell base reveal multiple focal adhesions at the edges of control cells with only modest FAK-paxillin co-localization. **Upper panels,** arrow shows a focal adhesion demonstrating clear co-localization. Following HGF stimulation, fewer total focal adhesions were detected with apparent condensation of focal adhesions at the tip of newly forming processes (middle panels). These areas of focal adhesion condensation revealed the greatest degree of FAK-paxillin co-localization. Pretreatment with U0126 prevented the process formation and focal adhesion condensation (lower panels).

**Paxillin Associates with ERK and Is a Substrate for ERK Phosphorylation—**The ability of HGF to induce ERK-paxillin association and subsequent ERK phosphorylation of paxillin was then examined. To determine whether HGF stimulation can induce ERK-paxillin association, mIMCD-3 cells were stimulated with HGF for 10 min followed by pull-down assay using GST-NH\(_{2}\)-paxillin, a GST fusion protein containing amino-terminal paxillin amino acids 1–323, excluding the carboxy-terminal LIM domains. In cells stimulated with HGF, GST-NH\(_{2}\)-paxillin associated with phosphorylated ERK, whereas equal amounts of a control GST construct failed to bring down active ERK (Fig. 3A, left panel). Immunoprecipitation of native paxillin from HGF-stimulated mIMCD-3 cells confirmed the observation that HGF stimulation results in the association of paxillin and phosphorylated ERK2 with a lesser association detected between paxillin and phosphorylated ERK1 (Fig. 3A, right panel).

The possibility that this association of activated ERK and paxillin results in paxillin phosphorylation was investigated by immunoprecipitation of paxillin from mIMCD-3 cells followed by an in vitro kinase assay with purified active ERK2. In agreement with the results of Ku and Meier (26) who have recently shown that ERK can phosphorylate paxillin in thymoma cells, paxillin immunoprecipitated from control
mIMCD-3 cells was heavily phosphorylated by ERK in vitro (Fig. 3B, lane 3). In contrast, paxillin immunoprecipitated from cells that had been stimulated with HGF exhibited an 80% decrease in the level of in vitro phosphorylation by purified active ERK (Fig. 3B, lane 1, quantitated in Fig. 3C), suggesting that the ERK phosphorylation sites on paxillin were already occupied. This decreased in vitro phosphorylation of paxillin by ERK following HGF stimulation in vivo was prevented by pretreatment of the cells with U0126 (Fig. 3B, lane 2), demonstrating that it is dependent on ERK activation in the intact cells. Immunoblotting of the membrane with anti-paxillin was performed to determine the amount of paxillin immunoprecipitated (Fig. 3B, lower panel). Of note, the HGF-induced gel retardation of paxillin is not seen in this immunoblot due to the shorter time of electrophoresis of the radioactive gel. These data are most consistent with an HGF-mediated ERK-dependent phosphorylation of paxillin in vivo.

An examination of the sequence of paxillin using the ScanSite protein motif identification program (27) reveals several probable ERK phosphorylation sites contained in the NH2 terminus of the protein. To demonstrate whether ERK can phosphorylate the amino terminus of paxillin, an in vitro kinase assay was performed with GST-NH2-paxillin as substrate. ERK incubation with GST-NH2-paxillin induced in vitro phosphorylation of the protein detectable within 1 min by both autoradiography and retardation of gel mobility (Fig. 3D). By 30–60 min, essentially all of the paxillin had undergone a mobility shift, and a third band of even slower gel mobility, presumably representing a second phosphorylation site, had appeared.
**HGF Induces ERK-dependent Association of Paxillin and FAK**—The potential ERK phosphorylation sites in paxillin flank the LD2 and LD4 domains that mediate association of paxillin with FAK (28, 29). Therefore, we examined whether HGF could regulate the association of FAK and paxillin. Immunoprecipitation of paxillin from subconfluent mIMCD-3 cells followed by immunoblotting with FAK revealed that in control cells there was a low level of FAK in anti-paxillin immunoprecipitates, whereas following stimulation with HGF there was a marked increase in the FAK-paxillin association (Fig. 4A, left panel). The same result was detected in paxillin immunoblots of anti-FAK immunoprecipitates from subconfluent mIMCD-3 cells (Fig. 4B). Interestingly, in fully confluent mIMCD-3 cells, the level of FAK-paxillin association was increased compared with that seen in subconfluent cells, and under these conditions HGF stimulation caused only a modest increase in FAK-paxillin association (data not shown). The dependence of the HGF-mediated association of paxillin and FAK on cell confluence suggests that cell-cell contact might be an independent regulator of this process.

To determine whether the HGF-mediated increase in paxillin-FAK association required activation of the MAPK pathway, we examined FAK-paxillin co-immunoprecipitation in mIMCD-3 cells stimulated with HGF in the presence of U0126. In both anti-paxillin (Fig. 4A, left panel) and anti-FAK (Fig. 4B) immunoprecipitates, the inhibition of HGF-dependent ERK activation eliminated the HGF-stimulated increase in paxillin-FAK association. The possibility that ERK-mediated paxillin phosphorylation was directly responsible for the HGF-dependent increase in paxillin-FAK association was examined using GST-NH₂-paxillin that had been phosphorylated by ERK in vitro to perform pull-down assays from unstimulated mIMCD-3 cell lysates. Although non-phosphorylated GST-NH₂-paxillin was capable of associating with FAK in these experiments, there was a marked increase in the pull-down assay of FAK when GST-NH₂-paxillin had been first phosphorylated using active ERK (Fig. 4A, right panel).

Because HGF can induce tyrosine phosphorylation of FAK (Fig. 1) (5, 6) and paxillin-FAK association has been shown to result in the recruitment of FAK to focal adhesion complexes where it is activated, we also examined the possibility that HGF-mediated paxillin-FAK association is necessary for FAK phosphorylation. The inhibition of paxillin-FAK association by pretreatment with U0126 was found to diminish the HGF-induced tyrosine phosphorylation of FAK observed in newly plated mIMCD-3 cells (Fig. 4C). Thus, HGF induces ERK-dependent association of paxillin and FAK, and this association appears to be important for the HGF-dependent tyrosine phosphorylation of FAK. In contrast, the HGF-dependent tyrosine phosphorylation of paxillin was not inhibited by U0126 (Fig. 2B), suggesting that a tyrosine kinase other than FAK is mediating this phosphorylation. Indeed, preliminary experiments with the src inhibitor PP1 reveal marked inhibition of the HGF-dependent paxillin tyrosine phosphorylation (data not shown).

**HGF-induced Cell Spreading and Adhesion Are ERK-dependent**—We have recently demonstrated that HGF mediates more rapid adhesion of mIMCD-3 cells on several substrates including fibronectin, type I collagen, and plastic (8). The potential importance of FAK in this phenotype has been demonstrated by Richardson et al. (30) who have shown that expression of FAK-related non-kinase inhibits paxillin tyrosine phosphorylation and cell spreading as well as Lai and coworkers (31) who have shown that the expression of FAK-related non-kinase inhibits HGF-induced Madin-Darby canine kidney cell spreading and migration. To investigate whether HGF-mediated cell adhesion involves enhanced cell spreading and whether regulation of the paxillin-FAK association is important for this phenotype, we examined the effect of inhibition of ERK activation on HGF-dependent mIMCD-3 cell adhesion. mIMCD-3 cells plated for 30 min in the presence of HGF exhibited a 32% increase in adhesion as compared with control cells, an effect that was completely inhibited by pretreatment with U0126 (Fig. 6A). Microscopy of the adherent cells revealed that cells treated with HGF had begun to flatten and spread as compared with control cells, and that the inhibition of ERK activation prevented HGF-mediated cell spreading (Fig. 6B). These results are consistent with our prior observation that HGF does not alter initial cell attachment at 10 min but rather induces increased adhesion at 30 and 60 min (the time when cell spreading occurs) and suggest that ERK-mediated paxillin-FAK association is likely to be an important determinant of HGF-dependent epithelial cell spreading and adhesion.

In conclusion, we have made the novel observation that HGF induces not only an increase in tyrosine phosphorylation of the focal adhesion complex protein paxillin but that paxillin is also phosphorylated in an ERK-dependent manner and this phosphorylation regulates the association of paxillin and FAK. This ERK-dependent regulation of paxillin in turn appears to be critical for the HGF-mediated tyrosine phosphorylation of FAK and for HGF-induced cell spreading and adhesion.

**REFERENCES**

1. Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimoniishi, M., Sugimura, A., Tashiro, K., and Shimizu, S. (1989) *Nature* 342, 440–443
2. Sonnenberg, E., Meyer, D., Weidner, K. M., and Birchmeier, C. (1993) *J. Cell Biol.* 123, 223–235
3. Montesano, R., Matsumoto, K., Nakamura, T., and Orci, L. (1991) *Cell* 67, 901–908
4. Cantley, L. G., Barros, E. J., Gandhi, M., Rauchman, M., and Nigam, S. K. (1994) *Am. J. Physiol.* 267, F271–F280
5. Nebe, B., Sunthuhen, H., Pommerenke, H., Peters, A., and Rychly, J. (1998) *Exp. Cell Res.* 243, 263–273
6. Bevilacqua, L., and Kramer, R. H. (1999) *Int. J. Cancer* 83, 640–649
7. Trusolino, L., Cavassa, S., Angelini, P., Ando, M., Bertotti, A., Comoglio, P. M., and Bocaccio, C. (2000) *FASEB J.* 14, 1629–1640
8. Liu, Z. X., Nickel, C. H., and Cantley, L. G. (2001) *Am. J. Physiol.* 281, F62–F70
9. Chen, H. C., Chan, P. C., Tang, M. J., Cheng, C. H., and Chang, T. J. (1998) *J. Biol. Chem.* 273, 25777–25782
10. Rauchman, M., Nigam, S., Delpriere, E., and Gullans, S. (1993) *Am. J. Physiol.* F416–F424
11. Liu, S., Thomas, S. M., Woodside, D. G., Rose, D. M., Kiosses, W. B., Pfaff, M., and Ginsberg, M. H. (1999) *Nature* 402, 676–681
12. Roshan, B., Kjeldsberg, C., Spikes, K., Eldred, A., Crovello, C. S., and Cantley, L. G. (1999) *J. Biol. Chem.* 274, 36362–36368
13. Oliver, M. H., Harrison, N. K., Bishop, J. E., Cole, P. J., and Laurent, G. J. (1989) *J. Cell Sci.* 92, 513–518
14. Bellia, S. L., Perrotta, J. A., Curtis, M. S., and Turner, C. E. (1997) *Biochem. J.* 325, 375–381
15. Turner, C. E. (1998) *Int. J. Biochem. Cell Biol.* 30, 955–959
16. Turner, C. E., Glenney, J. R., Jr., and Burridge, K. (1990) *J. Cell Biol.* 111, 1059–1068
17. Brown, M. C., Perrotta, J. A., and Turner, C. E. (1998) *Mol. Biol. Cell* 9, 1803–1816
18. Zachary, I., Sinnett-Smith, J., Turner, C. E., and Rezengurt, E. (1993) *J. Biol. Chem.* 268, 22060–22065
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19. Rankin, S., and Rozengurt, E. (1994) *J. Biol. Chem.* **269**, 704–710

20. Melamed, I., Turner, C. E., Aktories, K., Kaplan, D. R., and Gelfand, E. W. (1995) *J. Exp. Med.* **181**, 1071–1079

21. Turner, C. E., Pietras, K. M., Taylor, D. S., and Molloy, C. J. (1995) *J. Cell Sci.* **108**, 333–342

22. Schutkowski, M., Bernhardt, A., Zhou, X. Z., Shen, M., Reimer, U., Rahfeld, J. U., Lu, K. P., and Fischer, G. (1998) *Biochemistry* **37**, 5566–5575

23. Karihaloo, A., O’Rourke, D. A., Nickel, C., Spokes, K., and Cantley, L. G. (2001) *J. Biol. Chem.* **276**, 9166–9173

24. Davies, S. P., Reddy, H., Caivano, M., and Cohen, P. (2000) *Biochem. J.* **351**, 95–105

25. Yu, C. F., Roshan, B., Liu, Z. X., and Cantley, L. G. (2001) *J. Biol. Chem.* **276**, 32552–32558

26. Ku, H., and Meier, K. E. (2000) *J. Biol. Chem.* **275**, 11333–11340

27. Yaffe, M. B., Leparc, G. G., Lai, J., Obata, T., Volinia, S., and Cantley, L. C. (2001) *Nature Biotechnol.* **19**, 348–353

28. Brown, M. C., Perrotta, J. A., and Turner, C. E. (1996) *J. Cell Biol.* **133**, 1109–1123

29. Ku, H., and Meier, K. E. (2000) *J. Biol. Chem.* **275**, 11333–11340

30. Tachibana, K., Sato, T., D’Avirro, N., and Morimoto, C. (1995) *J. Exp. Med.* **182**, 1089–1099

31. Richardson, A., Malik, R. K., Hildebrand, J. D., and Parsons, J. T. (1997) *Mol. Cell. Biol.* **17**, 6906–6914

32. Lai, J. F., Kao, S. C., Jiang, S. T., Tang, M. J., Chan, P. C., and Chen, H. C. (2000) *J. Biol. Chem.* **275**, 7474–7480