Phosphatidylinositol-3 Kinase Is Necessary for 12-O-Tetradecanoylphorbol-13-acetate-induced Cell Transformation and Activated Protein 1 Activation

(Received for publication, August 5, 1996, and in revised form, November 26, 1996)

Chuanshu Huang, Patricia C. Schmid, Wei-Ya Ma, Harald H. O. Schmid, and Zigang Dong‡
From The Hormel Institute, University of Minnesota, Austin, Minnesota 55912

Phorbol esters, which activate isomers of protein kinase C, are general activators of the transcription factor activated protein 1 (AP-1). The pathway involved in this signal transduction is not very clear. Currently, little is known about whether phosphatidylinositol-3 (PI-3) kinase plays any role in phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced signal transduction. We demonstrate here that TPA not only has markedly synergistic effects on insulin-induced PI-3 kinase activity, but it also can induce PI-3 kinase activity and the PI-3 phosphates by itself. We also found that insulin, a PI-3 kinase activator, enhanced TPA-induced AP-1 trans-activation and transformation in JB6 promotion-sensitive cells. Furthermore, wortmannin and LY294002, two PI-3 kinase inhibitors, markedly decreased AP-1 activity induced by insulin, TPA, or TPA and insulin and inhibited JB6 promotion-sensitive cell transformation. All evidence from present studies suggests that PI-3 kinase acts as a mediator in TPA-induced AP-1 activation and transformation in JB6 cells.

Phosphatidylinositol-3 imaging plays a central role in a broad range of biological effects (1–4). This enzyme is a dimer composed of a catalytic subunit (P110) and a regulatory subunit (P85) (5). The P85 regulatory subunit has no discernible catalytic activity but possesses two Src homology 2 domains and an Src homology 3 domain (6). A region between the two Src homology 2 domains of P85 binds the NH2 terminus of P110, mediating the constitutive association of the two subunits (6). Binding of P85 to P110 partially activates P110 (7, 8). PI-3 kinase phosphorylates the lipid PI on the 3 position of the α-myo-ositol ring, yielding PI-3-phosphate (9). Because the enzyme can also use phosphorylated forms of phosphatidylinositol (PI-4-phosphate and PI-4,5-bisphosphate) as substrates, activation of the PI-3 kinase also leads to the generation of PI-3,4-P2 and PI-3,4,5-triphosphate (5, 9, 10). Previous studies suggested that these PI-3 kinase products are potential second messengers (1, 12, 13).

12-O-Tetradecanoylphorbol-13-acetate (TPA) is not only a potent tumor promoter in mouse skin (14, 15), but it also induces a wide range of other biological effects in cultured cells (16). Protein kinase C (PKC) is well known as a TPA receptor and a phospholipid-dependent kinase involved in basic cellular functions, including regulation of cell growth, differentiation, and gene expression (17, 18). PKC isozymes include more than 11 different enzymes (α, βI, βII, γ, δ, ε, ζ, η, θ, λ, and μ). Previous studies indicated that the TPA-induced activation of AP-1, NFκB, and other transcription factors in the nucleus is mediated by the Ras-Raf/1-MAP kinase cascade (18–20). The role of PI-3 kinase in the TPA-induced signal transduction pathway, however, is not clear, even though some reports indicated that PI-3 kinase and p21ras modulate each other’s signals (2, 4, 21). For example, overexpression of activated p21ras in PC12 cells increases PI-3 kinase activity and stimulates the accumulation of 3’-phosphorylated inositol lipids in the cells (21). GTP-bound p21ras also directly binds and activates PI-3 kinase in vitro (21). Expression of activated PI-3 kinase in NIH 3T3 cells apparently potentiates p21ras-dependent signaling events (2). Furthermore, PI-3 kinase products may play an important role in extensive cross-talk among multiple signaling pathways and regulation of cell function (1, 12, 13). Since both phorbol ester- and insulin-induced activation of the Ras-Raf/1-MAP kinase pathway and a combination of insulin and phorbol ester resulted in a synergistic activation of this pathway (22), we inquired whether TPA can activate PI-3 kinase and whether activation of PI-3 kinase is required for TPA-induced signal transduction and cell transformation. In the present study, we used several approaches, which included a PI-3 kinase activator, two pharmacological PI-3 kinase inhibitors, and a dominant negative PI-3 kinase inhibitor, to study the role of PI-3 kinase in TPA-induced AP-1 activation and cell transformation in the well-characterized mouse epidermal JB6 cells.

MATERIALS AND METHODS

Plasmids and Reagents—The AP-1 luciferase reporter plasmid (–73/+63 collagenase-luciferase) and cytomegalovirus-neo marker vector plasmid were as previously reported (23); the bovine PI-3 kinase P85 subunit mutant plasmid (ΔP85) and vector plasmid SRE were as described by Hare et al. (24); agarose conjugated with mono-clonal antiphosphotyrosine antibody Py20 was from Santa Cruz Bio-technology; fetal bovine serum (FBS) was from Life Technologies, Inc.; LipofectAMINE was from Life Technologies, Inc.; Eagle’s minimal essential medium (MEM) and wortmannin were from Calbiochem; LY294002 was from Biomek; TPA was from Calbiochem; insulin was from Sigma; luciferase assay substrate was from Promega; and γ-32P/ATP was from DuPont NEN.

Cell Culture—The JB6 P+ mouse epidermal cell line C1 41 and the stable AP-1 luciferase reporter plasmid transfected mouse epidermal...
PI-3 Kinase and TPA-induced Cell Transformation and AP-1 Activation

JB6 P* cell line C1 41-19 (25, 26) were cultured in monolayers at 37 °C in 5% CO2 using Eagle’s minimal essential medium containing 5% fetal calf serum, 2 mM l-glutamine, and 25 µg of gentamicin/ml.

Generation of Stable Cotransfectants with AP-1 Reporter and Dominant Negative PI-3 Kinase Mutant—JB6 P* cells, C1 41, were cultured in a 6-well plate until they reached 85–90% confluence. We used 2 µg of AP-1 luciferase reporter plasmid and 0.3 µg of cytomegalovirus-neo vector with 6 µg of a dominant negative mutant of PI-3 kinase P85 subunit plasmid ΔP85 or vector SRa control plasmid DNA and 15 µl of LipofectAMINE reagent to transfect each well in the absence of serum. After 10–12 h, the medium was replaced by 5% FBS and MEM. Approximately 30–36 h after the beginning of the transfection, the cells were trypsinized, and the cell suspension was seeded into 75-ml culture flasks and cultured for 24–28 days with G418 selection (300 µg/ml). Stable transfectants were screened by assay of the luciferase activity and Western blotting with rabbit polyclonal IgG against human PI-3 kinase P85α. Stable transfected cells, ΔP85 mass1, ΔP85 mass2, and AP-1 mass1, were cultured in G418-free MEM for at least two passages before each experiment.

Assay for AP-1 Activity—Confluent monolayers of JB6 C1 41–19, ΔP85 mass1, ΔP85 mass2, or AP-1 mass1 cells were trypsinized, and 5 x 10^5 viable cells suspended in 100 µl 5% FBS and MEM were added into each well of a 96-well plate. Plates were incubated at 37 °C in a humidified atmosphere of 5% CO2. Twelve to 24 h later, cells were stained by culturing them in 0.1% BME solution for 30 min at 37 °C before exposure to TPA or insulin. The cells were exposed to TPA, insulin, and TPA and insulin for AP-1 induction with or without different concentrations of wortmannin or LY294002 for 24 h. The cells were extracted with lysis buffer, and luciferase activity was measured using a luminometer (Monolight 2010). The results are expressed as the relative AP-1 activity or relative luciferase units (28).

PI-3 Kinase Assay—PI-3 kinase activity was assayed according to the method of Endemann et al. (27). In brief, JB6 cells, C1 41, ΔP85 mass1, and ΔP85 mass 2 or AP-1 mass1, were cultured in monolayers in 100-mm plates. Then cells were incubated in 0.1% FBS and MEM for 24 h and in serum-free MEM for 3–4 h at 37 °C, respectively. TPA (10 ng/ml) with or without wortmannin was added, and after 20 min at 37 °C, 2.5 µg/ml insulin was added. After 10 min at 37 °C, the cells were washed once with ice-cold phosphate-buffered saline and lysed in 400 µl of lysis buffer/plate (20 mM Tris, pH 8.3, 137 mM NaCl, 1 mM MgCl2, 10% glycerol, 1% Nonidet P-40, 1% dithiothreitol, 0.4 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride). The lysates were centrifuged, and the supernatants were incubated overnight at 4°C with 100 µg of antibody to PI-3 kinase, and after 20 min at 37 °C, the antibodies were removed by washing the lysates four times. Then each of the following buffers: 1) phosphate-buffered saline with 1% Nonidet P-40, 1% dithiothreitol; 2) 0.1 M Tris, pH 7.6, 0.5 M LiCl, 1 mM dithiothreitol; and 3) 10 mM Tris, pH 7.6, 0.1 M NaCl, 1 mM dithiothreitol. The beads were incubated for 5 min on ice in 20 µl of buffer 3, and then 20 µl of 0.5 mg/ml phosphatidylinositol (previously sonicated in 50 mM HEPES, pH 7.6, 0.1 M EGTA, 1 mM Na2HPO4) was added. After 5 min at the same temperature, 10 µl of the reaction buffer was added (50 mM MgCl2, 100 mM HEPES, pH 7.6, 250 µM ATP containing 5 µCi of [γ-32P]ATP), and the beads were incubated for an additional 5 min. The reactions were stopped by the addition of 15 µl of 4 N HCl and 130 µl of chloroform:methanol (1:1). After vortexing for 30 s, 30 µl from the phospholipid-containing chloroform phase was spotted onto thin-layer chromatography plates coated with Silica Gel H containing 1.5% water. After drying, the plates were developed in a solvent system consisting of 70% methanol and 20% water for 254 nm.

Intracellular PI-3,4-P2 Assay—JB6 C1 41 cells were cultured in monolayers in 100-mm dishes. The cells were washed three times with phosphate-free MEM and starved in the same medium for 24 h. Before stimulation, the cell medium was changed to fresh phosphate-free MEM and insulin was added at 2.5 µg/ml for another 10 min. The cells were harvested, and the PI-3 kinase was immunoprecipitated with monoclonal antibody PY20. The immunoprecipitates were incubated with PI and [γ-32P]ATP. The phospholipids were separated by TLC. The TLC plates were dried at room temperature and autoradiographed. PI-3,4-P2, PI-3-phosphate.

TPA induces PI-3 kinase activity and enhances insulin-induced PI-3 kinase activity. JB6 C1 41 cells were treated with medium or TPA (10 ng/ml) with or without wortmannin for 20 min at 37 °C in 5% CO2. Cells were then exposed to insulin (2.5 µg/ml) for another 10 min. The cells were harvested, and the PI-3 kinase was immunoprecipitated with monoclonal antibody PY20. The immunoprecipitates were incubated with PI and [γ-32P]ATP. The phospholipids were separated by TLC. The TLC plates were dried at room temperature and autoradiographed. PI-3,4-P2, PI-3-phosphate.

TPA Induces PI-3 Kinase Activity and Enhances Insulin-induced PI-3 Kinase Activity—To determine whether PI-3 kinase plays any role in TPA-induced signal transduction, we first investigated whether TPA can induce PI-3 kinase activity. We analyzed PI-3 kinase in JB6 P* cells. Cells (1 x 10^9/ml) were exposed to TPA or insulin with or without wortmannin or LY294002 in 1 ml of 0.5% BME agar containing 10% FBS. The plates were cultured in a 37 °C, 5% CO2 incubator for 14–21 days, and the cells were scored by the methods described by Colburn et al. (32). The effect of wortmannin or LY294002 on transformation of JB6 cells was presented as a percentage inhibition of cell transformation.

RESULTS

TPA Induces PI-3 Kinase Activity and Enhances Insulin-induced PI-3 Kinase Activity

TPA induces PI-3 kinase activity and enhances insulin-induced PI-3 kinase activity. To determine whether PI-3 kinase plays any role in TPA-induced signal transduction, we first investigated whether TPA can induce PI-3 kinase activity. We analyzed PI-3 kinase in JB6 P* cells stimulated by TPA. As shown in Fig. 1, TPA not only had a synergistic effect on insulin-induced PI-3 kinase activity, but it also induced PI-3 kinase activity by itself. Induction or enhancement of PI-3 kinase activity by TPA could be blocked by wortmannin (Fig. 1). To test whether the PI-3 phosphates were increased in the cells stimulated by TPA, we also measured the products of PI-3 kinase in TPA-treated JB6 cells by HPLC. The results show that PI-3,4-P2 in JB6 cells was increased after cells were stimulated by insulin or TPA (Fig. 2). PI-3-phosphate and PI-3,4,5-triphosphate could not be clearly identified.
nin (100 nM) inhibited AP-1 activity induced by insulin, TPA, or TPA and insulin by 92.0, 50.0, or 57.3%, respectively (Fig. 3).

Inhibition of TPA-induced AP-1 Activity in JB6 P<sub>1</sub> Cells by Wortmannin—Wortmannin inhibits PI-3 kinase by covalently binding to P110 of the enzyme. Unlike wortmannin, LY294002 inhibits PI-3 kinase by competing with ATP for its substrate binding site (33). We therefore used this other PI-3 kinase inhibitor, LY294002, to determine the role of the PI-3 kinase in AP-1 trans-activation induced by insulin or TPA. The results showed that LY294002 inhibited AP-1 activity induced by insulin or TPA in a dose-dependent manner (Fig. 4).

Insulin Induces AP-1 Trans-activation and Enhances TPA-induced AP-1 Activity—Previous studies demonstrated that insulin or TPA induced partial activation of the extracellular signal-regulated protein kinase, whereas a combination of insulin and TPA resulted in a synergistic activation of the extracellular signal-regulated protein kinase in Rat-1 HIR cells (22). To investigate whether insulin could induce AP-1 or promote TPA-induced AP-1 activity in JB6 cells, we exposed JB6 P<sup>+</sup> cells to insulin, TPA, or TPA and insulin. The results showed that insulin could markedly induce AP-1 activity and increase TPA-induced AP-1 activity in JB6 P<sup>+</sup> cells in a 0.1% FBS medium culture system (Fig. 5A). The induction of AP-1 activity and the increase in TPA-induced AP-1 activity by insulin occur in a dose-dependent manner (Fig. 5B). At a serum concentration higher than 0.25%, the increase of TPA-induced AP-1 activity by insulin could not be observed (Fig. 6).

Overexpression of the Dominant Negative PI-3 Kinase P85<sub>a</sub> Blocks Insulin- or TPA-induced AP-1 Activity—Since significant inhibition of insulin- or TPA-induced AP-1 activity was achieved by using both PI-3 kinase inhibitors, wortmannin and LY294002, PI-3 kinase may play a critical role in TPA- or insulin-induced AP-1 activation. The dominant negative mutant of PI-3 kinase, ΔP85, has been shown to be a specific inhibitor of PI-3 kinase in Chinese hamster ovary cells (24). To specifically block PI-3 kinase and to test the role of PI-3 kinase in TPA-induced AP-1 activation in JB6 cells, the dominant negative mutant of the PI-3 kinase regulatory subunit P85 plasmid and AP-1 reporter were cotransfected into JB6 cells by using a LipofectAMINE kit. Three stable mass cultures, two (ΔP85 mass1 and ΔP85 mass2) from cotransfection AP-1 reporter and vector SRo, were established by G418 selection (34). To determine whether overexpression of the dominant negative PI-3 kinase protein blocks TPA- or TPA- and insulin-induced PI-3 kinase activity, we also tested the PI-3 kinase activity in ΔP85 mass1 and mass2 cells induced by insulin, TPA, or TPA and insulin. The results show that the PI-3 kinase activity induced by insulin, TPA, or TPA and insulin was completely blocked by expression of the dominant negative PI-3 kinase protein (Fig. 7). Furthermore, overexpression of the dominant negative PI-3 kinase protein in ΔP85 mass1 and ΔP85 mass2 cells blocked insulin- or TPA-stimulated AP-1 activity completely compared with that in AP-1 mass1 cells in all the sample points of the time and dose courses studied (Figs. 8 and 9).

Enhancement by Insulin of TPA-induced JB6 P<sup>+</sup> Cell Trans-
Since our previous results and other studies demonstrated that induced AP-1 activity is important and required for cell transformation, we tested whether insulin could induce transformation or promote TPA-induced transformation. The results showed that insulin could not induce JB6 P+ cell transformation alone; however, it markedly increased the TPA-induced JB6 P+ cell transformation rate (Fig. 10).

**DISCUSSION**

Our present studies demonstrate that PI-3 kinase is a crucial mediator of TPA-induced cell transformation and AP-1 trans-activation in JB6 cells. TPA alone could induce PI-3 kinase activity and increase the level of PI-3,4-P2 in JB6 cells. More interestingly, TPA and insulin synergistically induced PI-3 kinase activity. Insulin, a strong PI-3 kinase activator, enhanced TPA-induced AP-1 activation and cell transformation. Furthermore, wortmannin and LY294002, two different kinds of PI-3 kinase inhibitors, which inhibit PI-3 kinase by interfering with P110 and P85, respectively, inhibit TPA-induced AP-1 activation as well as cell transformation. More convincingly, TPA- and TPA- and insulin-induced AP-1 activation and TPA-induced cell transformation could be blocked completely by overexpression of the dominant negative PI-3 kinase P85 mutant. AP85 mass1 and AP85 mass2, were almost totally blocked, whereas the AP-1 mass1 cells showed a high frequency of the transformation rate with exposure to TPA.

Activation of PKC requires both association with the membrane and a number of activators and cofactors, the requirements for which differ for each isozyme (35). Thus, PKCs are grouped into three major classes: conventional PKC isoforms,
such as \( \alpha, \beta I, \beta II, \) and \( \gamma \); novel PKCs, including \( \delta, \epsilon, \eta, \) and \( \theta; \) and atypical PKCs, represented by the \( \zeta \) and \( \lambda \) isoforms of PKC (35, 36). Activation of atypical PKCs could be carried out by either the PI-3 kinase pathway or the ceramide pathway (37, 38). Conventional PKCs are activated by diacylglycerol in a \( \text{Ca}^{2+} \)-dependent manner. In contrast, activation of novel PKCs is \( \text{Ca}^{2+} \)-independent (35, 36). In addition to the natural activator, conventional PKCs and novel PKCs are also activated with high specificity by TPA (17). For this reason, TPA is often used in the study of the mechanisms of conventional PKC and novel PKC activation and their function. Most of the previous studies have addressed the regulation of phosphorylation of the insulin receptor by PKC in PKC isozyme-transfected cells (39, 40). Overexpression of PKC isozymes \( \alpha, \beta I, \gamma, \) and \( \epsilon \) did not affect expression of the insulin receptor or insulin-stimulated tyrosine phosphorylation of the receptor. However, in response to phorbol esters, cells expressing PKC \( \alpha, \gamma, \) and \( \beta I \) but not \( \epsilon \) exhibited 3–4-fold higher levels of insulin receptor (IR) phosphorylation. This TPA-stimulated IR phosphorylation inhibits the activation of the insulin receptor kinase and the insulin-induced PI-3 kinase activity as well as the tyrosine phosphorylation of Shc (39, 40), but this inhibition is not observed in the cells containing only the endogenous levels of PKC (40). In the present study, we demonstrated that TPA induces a low level of PI-3 kinase activity and has significant synergistic effects with insulin on activation of PI-3 kinase in mouse epidermal JB6 cells. The reason for the difference among data from different cells may be due to different levels of endogenous PKC in the various cell types studied as well as differences in ratios of various PKC isozymes present in different cells. The ratio of PKC:IR in different cells may be another reason for these differences.

Several studies suggested that the PI-3 kinase and its products PI-3,4-P₂ and PI-3,4,5-triphosphate are important regulators of cell proliferation and \( c-fos \) gene expression (1–3). The introduction of the NH₂-terminal Src homology 2 domain of the P85 subunit of PI-3 kinase into cells abrogates the insulin- or
IGF-1-stimulated DNA synthesis and prevents insulin stimulation of c-Fos protein expression (2, 3). The microinjection of a dominant-negative p21ras mutant or anti-Ras antibody inhibited insulin-induced DNA synthesis (3). A constitutively activated mutant P110 induced transcription from the Fos promoter; coexpression of dominant negative Ras blocked this response (2). Other studies have shown that PI-3,4-P2 and PI-3,4,5-triphosphate are elevated in cells transformed by v- abl, v-src, and polyoma middle T, and decreased levels of these lipids correlate with impaired cell transformation by mutated forms of these oncogenes (1, 41, 42). It has been reported that the presence of insulin-like growth factor I receptor (IGF-IR) is an obligatory requirement for the establishment and maintenance of the tumor phenotype (43–46). Cells derived from mouse embryos with a targeted disruption of the IGF-IR gene (R2 cells) cannot be transformed by SV40 T antigen or by an activated and overexpressed Ha-ras, even by a combination of both, all of which transform very efficiently the corresponding wild type cells or other 3T3-like cells (47). If a plasmid expressing a wild type human IGF-1 receptor cDNA is stably transfected into R2 cells, the cells can be transformed by SV40 T antigen. This indicates that the defect in transformability is specifically due to the lack of IGF-IR (46). Substantial evidence has been reported that PI-3 kinase is a critical component of signaling pathways used by the cell surface receptors for a variety of mammalian growth factors or other stimulators (1, 10, 12, 48), especially IR and IGF-IR. Recently, it was reported that insulin could activate the Ras-Raf/MAP kinase pathway by interacting and activating its receptors (7). Dhand et al. (7) suggested that activation of this Ras/MAP kinase pathway is critical for the effect of insulin on mitogenesis and c-fos expression. Others found that neither insulin nor phorbol ester regulated phosphatidylinositolcarboxykinase gene expression requires activation of the Ras/MAP kinase pathway, but PI-3 kinase is required in this event (49). In contrast, Sakaue et al. (50) demonstrated that neither the Ras/MAP kinase cascade nor PI-3 kinase may be required for insulin-stimulated glycosynthase activation in Chinese hamster ovary cell lines. Evidence from different groups using different model systems has confirmed the crucial importance of AP-1 activity in transformation and carcinogenesis (23, 26, 51, 53). Our previous results have shown that AP-1 activity is required for tumor promotion in the JB6 cell model (23, 26, 52, 53). High basal levels of AP-1 activity appear to be important for the maintenance of tumor phenotypes in the transformed cell line RT101 (52, 53). Since our primary results (Fig. 1) showed that TPA can induce PI-3 kinase activity and increase the level of PI-3,4-P2 (Fig. 2), as well as have a markedly synergistic effect with insulin on PI-3 kinase activation, a critical question is whether PI-3 ki-
PI-3 Kinase and TPA-induced Cell Transformation and AP-1 Activation

The two pharmacological inhibitors (wortmannin and LY294002) and the biological inhibitor (ΔP85, a dominant negative mutant of the PI-3 kinase P85 subunit of PI-3 kinase) markedly blocked TPA-induced AP-1 activation and cell transformation. Specific blockage of the events required for cell transformation with few side effects on normal growth might be a promising target for cancer prevention and treatment. In fact, inhibiting induced PI-3 kinase and AP-1 activity by a pharmacological inhibitor (wortmannin) or a dominant negative mutant of PI-3 kinase (ΔP85) does not seem to have inhibitory effects on cell growth in JB6 cells. Further investigation of this topic may elucidate the precise mechanisms underlying the role of PI-3 kinase in phorbol ester-induced signal transduction and may thus provide a novel target for the prevention of carcinogenesis.

Acknowledgments—We thank Dr. Masato Kasuga for the generous gift of the bovine PI-3 kinase P85 subunit mutant plasmid ΔP85, Dr. Vincent Duronio for supplying the protocol for HPLC analysis, and Jeannie Ruble for secretarial assistance.

REFERENCES

1. Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapellier, R., and Soltoff, S. (1991) Cell 64, 281–302; Correction (1991) Cell 65

2. Hu, Q., Klippel, A., Muslin, A. J., Fantl, W. J., and Williams, L. T. (1995) Science 268, 100–102

3. Jhan, B. H., Rosen, D. W., Seely, B. L., Ramey, L., Cantley, L., Saltiel, A. R., and Olefsky, J. M. (1994) Mol. Cell. Biol. 14, 7466–7475

4. Rodriguez-Viciana, P., Warne, P. H., Vanhaesebroeck, B., Waterfield, M. D., and Downward, J. (1996) EMBO J. 15, 2442–2451

5. Carpenter, C. L., Duckworth, B. C., Auger, K. R., Cohen, B., Schaffhausen, B. S., and Cantley, L. C. (1990) J. Biol. Chem. 265, 19704–19711

6. Kapellier, R., and Cantley, L. C. (1994) Bioessays 16, 556–576

7. Dhand, R., Hara K., Hiles, I., Bax, B., Gout, I., Parayayotou, G., Fry, M. J., Yonezawa, K., Kasuga, M., and Waterfield, M. D. (1994) EMBO J. 13, 511–521

8. Hu, P., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 2577–2583

9. Whitman, M., Downes, C. P., Keeler, M., Keller, T., and Cantley, L. (1988) Nature 332, 644–646

10. Auger, K. R., Seruiania, L. A., Soltoff, S. P., Libby, P., and Cantley, L. C. (1989) Cell 77, 167–175

11. Schaffer, L. (1994) Eur. J. Biochem. 214, 1127–1132

12. Downes, C. P., and Carter, A. N. (1991) Cell Signal. 3, 501–513

13. Stephens, L. R., Jackson, T. R., and Hawkins, P. T. (1990) Biochim. Biophys. Acta 1179, 27–75

14. Berenblum, I. (1975) in Cancer, A Comprehensive Treatise (Becker, F. F., ed) pp. 323–344, Plenum Publishing, New York

15. Shalgi, T., Fisch, S. M., Weeks, C. E., Klein-Szanto, A. J. P., and Reiners, J. (1982) J. Cell. Biochem. 18, 99–119

16. Vlachos, C. J., and Waterfield, M. D. (1994) Biochem. J. 265, 399–408

17. Nishizuka, Y. (1988) Nature 332, 642–646

18. Arnold, R. S., and Newton, A. C. (1996) J. Biol. Chem. 271, 5905–5914

19. Schaap, D., van der Wal, J., Howe, L. R., Marshall, C. J., and van Blitterswijk, W. J. (1993) J. Biol. Chem. 268, 20132–20136

20. Troppmair, J., Bruder, J. T., Munoz, H., Lloyd, P. A., Kyriakis, J., Banerjee, P., Avruch, J., and Rapp, U. R. (1994) J. Biol. Chem. 269, 7030–7035

21. Dong, Z. G., Birrer, M. J., Watts, R. G., Matrisian, L. M., and Colburn, N. H. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 18134–18139
38. Divecha, N., and Irvine, R. F. (1995) Cell 80, 269–278
39. Chin, J. E., Dickens, M., Tavare, J. M., and Roth, R. A. (1993) J. Biol. Chem. 268, 6338–6347
40. Danielsen, A. G., Liu, F., Hosomi, Y., Shii, K., and Roth, R. A. (1995) J. Biol. Chem. 270, 21600–21605
41. Fukui, Y., Saltiel, A. R., and Hanafusa, H. (1991) Oncogene 6, 407–411
42. Varticovski, L., Daley, G. Q., Jackson, P., Baltimore, D., and Cantley, L. C. (1991) Mol. Cell. Biol. 11, 1107–1113
43. Baserga, R. (1995) Cancer Res. 55, 249–252
44. Coppola, D., Forberg, A., Miura, M., Sell, C., D’Ambrosio, C., Rubin, R., and Baserga, R. (1994) Mol. Cell. Biol. 14, 4588–4595
45. Miura, M., Surmacz, E., Burgaud, J. L., and Baserga, R. (1995) J. Biol. Chem. 270, 22639–22644
46. Surmacz, E., Sell, C., Swartek, J., Kato, H., Roberts, C. T., Leroith, D., and Baserga, R. (1995) Exp. Cell Res. 218, 370–380
47. Sell, C., Rubini, M., Rubin, R., Liu, J.-P., Etstratiadis, A., and Baserga, R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11217–11221
48. Hawkins, P. T., Jackson, T. R., and Stephens, L. R. (1992) Nature 358, 157–159
49. Gabbay, R. A., Sutherland, C., Gnudi, L., Kahn, B. B., O’Brien, R. M., Granner, D. K., and Flier, J. S. (1996) J. Biol. Chem. 271, 1890–1897
50. Sakaue, H., Hara, K., Noguchi, T., Matuzaki, T., Kotani, K., Ogawa, W., Yonezawa, K., Waterfield, M. D., and Kasuga, M. (1995) J. Biol. Chem. 270, 11304–11309
51. Alani, R., Brown, P., Binetruy, B., Dosaker, H., Rosenberg, R. K., Angel, P., Karin, M., and Birrer, M. J. (1991) Mol. Cell. Biol. 11, 6286–6295
52. Domann, F. E., Levy, J. P., Birrer, M. J., and Bowden, G. T. (1994) Cell Growth & Differ. 5, 9–16
53. Dong, Z. G., Lavrovsky, V., and Colburn, N. H. (1995) Carcinogenesis (Lond.) 16, 749–756
54. Yano, H., Nakanishi, S., Kimura, K., Hanai, N., Saitoh, Y., Fukui, Y., Nonomura, Y., and Matsuda, Y. (1993) J. Biol. Chem. 268, 25846–25856

PI-3 Kinase and TPA-induced Cell Transformation and AP-1 Activation