Biochemical Pharmacology, Vol. 58, pp. 279–284, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00089-1

Different Sensitivities of p42 Mitogen-Activated Protein Kinase to Phorbol Ester and Okadaic Acid Tumor Promoters among Cell Types

Gian Paolo Rossini, Cristina Pinna and Claudia Malaguti*
DIPARTIMENTO DI SCIENZE BIOMEDICHE, UNIVERSITÀ DI MODENA, 41100 MODENA, ITALY

ABSTRACT. The operational equivalence of different types of tumor promoters was studied by comparing immediate, early, and late effects of okadaic acid (OA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) on the phosphorylation state of p42 mitogen-activated protein kinase isoform (ERK2) in eight different cell lines. In normal human and mouse fibroblasts, both agents stimulated immediate/early (15–60 min) phosphorylation of ERK2. In mouse 3T3 cells, enhanced phosphorylation of ERK2 was detected only within the first hour of treatment with TPA but not with OA. The early response to both TPA and OA, in turn, was lost in another established cell line, the PNT2 prostate epithelial cells, where we could detect increased levels of phosphorylated ERK2 only after a 24-hr treatment with OA. When the effect of OA was evaluated in different PNT cell strains, we observed that their capacity to respond to this agent, by stabilizing phosphorylated forms of ERK2, was lost in less differentiated strains. In HeLa S_3 and HTC tumor cells, however, neither TPA nor OA treatment led to any detectable increase in ERK2 phosphorylation at any time point analyzed. We conclude that the effects of OA and TPA on the phosphorylation states of ERK2 could be related to the cell type, and that the operational equivalence between these two different tumor promoters is maximal in normal cells. BIOCHEM PHARMACOL **58**;2:279–284, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. okadaic acid; phorbol ester; tumor promoter; phosphorylation; cell proliferation

Reversible phosphorylation of proteins is a key molecular mechanism involved in the control of cell functioning and proliferation [1]. Protein kinases and phosphoprotein phosphatases, the enzymes catalyzing these two opposite reactions, represent the effectors of many signal transduction systems and also constitute the molecular targets of several tumor promoters [1–4]. Phorbol esters such as TPA† and OA are examples of such a heterogeneous group of chemical agents, as phorbol esters activate some isoforms of protein kinase C by substituting for the endogenous intracellular messenger diacylglycerol [2, 5], whereas OA binds and causes inhibition of type 1 and 2A serine/threonine PP1 and PP2A [3, 4, 6–8].

In spite of the different molecular mechanisms of functioning of these two types of agents, their overall effect on tumor promotion might be due to converging chains of events over some common target proteins. Thus, by increasing the extent of protein phosphorylation either by stimulating phosphate addition, as in the case of phorbol esters, or by slowing down its removal (OA), tumor promoters would affect the molecular mechanisms controlling cell functioning and proliferation [2–4].

ERK2 represents a widely studied member of the MAPK family, whose activation depends on phosphorylation of specific threonine and tyrosine residues [9–11]. The phosphorylation state of these residues, in turn, depends on the activity of mitogen-activated/extracellular signal-regulated protein kinase-activating kinase (MEK), the dual specificity protein kinase responsible for ERK phosphorylation [10–13], and on phosphoprotein phosphatases, including PP1, 2A and CL100, which catalyze removal of phosphates and hence inactivate ERKs [14]. The important roles ascribed to ERK2 in the control of cell proliferation [9–11] make this kinase a possible target molecule shared by OA and TPA through enhancement of its phosphorylated state. In order to gain further insight into the molecular basis of the operational equivalence between OA- and phorbol ester-type tumor promoters, we set up a comparative study to evaluate their effects on the phosphorylation state of ERK2 in normal, established, and tumor cell lines.

MATERIALS AND METHODS

Materials

Peroxydase-linked anti-rabbit immunoglobulin antibody and the ECL detection reagents were from Amersham. OA

* Corresponding author: Dr. Gian Paolo Rossini, Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Via Campi 287, 41100 Modena, Italy. Tel. 39.059.42.85.02; FAX 39.059.42.85.24; E-mail: rossini@unimo.it

† *Abbreviations:* TPA, 12-*O*-tetradecanoylphorbol-13-acetate; OA, okadaic acid; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; PP1 and PP2A, phosphoprotein phosphatase 1 and 2A; and ERK2, p42 mitogen-activated protein kinase isoform.

Received 22 September 1998; accepted 8 February 1999.

was obtained from Alexis Corporation (LC Laboratories) and TPA and prestained molecular mass markers from Sigma. The anti-ERK2 antibody was purchased from Santa Cruz Biotechnology. Protran BA83 nitrocellulose membrane was from Schleicher and Schuell. All other reagents were of analytical grade.

Cell Culture Conditions

HeLa S_3, HTC, and Swiss albino mouse 3T3 cells were obtained from the American Type Culture Collection. Normal human fibroblasts were obtained from explants of surgically discarded skin from normal subjects. PNT normal prostate epithelial cells [15] were obtained from Dr. Philippe Berthon (Departement d'Urologie, Hôpital St. Louis, Paris, France). Normal mouse fibroblasts were obtained from explants of skin from normal mice.

HTC and 3T3 cells and normal fibroblasts were grown in 5% carbon dioxide in air at 37°, in 90-mm diameter Petri dishes, with a culture medium composed of Dulbecco's modified Eagle's medium containing 1% non-essential amino acids and 10% foetal bovine serum, as previously described [16]. PNT normal prostate epithelial cells (strains PNT2, PNT1A, and PNT1B) and HeLa S_3 cells were grown as described above, but with a culture medium composed of RPMI 1640, containing 5% foetal bovine serum, as reported previously [15].

Preparation of Cell Lysates

Cells were washed three times with 20 mM phosphate buffer, pH 7.4, 0.15 M NaCl (PBS) and were subdivided into two aliquots, which were lysed by addition of either a hypotonic or solubilization buffer. In the first case, cells corresponding to 10–15% of original suspension were dispersed in 1 mL of 20 mM Tris–HCl, pH 7.5 at 2°, 1.5 mM EDTA, and were lysed by sonication at 2° with two 8-sec bursts at an output of 15 W. This total lysate was used to determine the DNA content of cell suspensions, using the procedure of Labarca and Paigen [17]. The second aliquot, corresponding to the remaining portion of original cell suspension, was lysed by dispersion in 0.5 mL of PBS containing 1% (v/v) Nonidet P-40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 50 mM NaF, 10 mM $Na_4P_2O_7$, 0.1 mg/mL phenylmethylsulphonyl fluoride, 1 mM Na_3VO_4, 30 μg/mL aprotinin, and by two 10-sec bursts of vortexing. Cytosoluble extracts were then obtained by centrifugation for 30 min at 16,000 × *g*. The supernatants of this centrifugation were used for colorimetric determinations of protein content with bicinchoninic acid [18], and were then brought to 2% SDS and 5% β-mercaptoethanol for use in fractionation by SDS-PAGE.

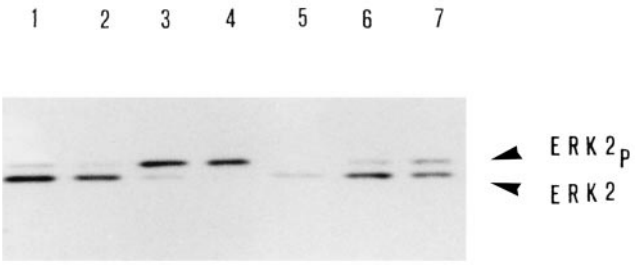

FIG. 1. Effect of OA and TPA treatment of normal human fibroblasts on the electrophoretic mobility of ERK2. Extracts from control cells (lane 1) and cells which had been treated with either 100 nM TPA (lanes 2–4) or 50 nM OA (lanes 5–7) were subjected to SDS-PAGE, with the same amounts of protein loaded onto each lane. After electrophoresis, samples were subjected to immunoblotting using anti-ERK2 antibody as described under Materials and Methods. Treatments of cultures with OA or TPA were carried out for 15 min (lanes 4 and 7), 1 hr (lanes 3 and 6), or 24 hr (lanes 2 and 5) at 37°. The electrophoretic mobilities of phosphorylated ($ERK2_p$) and dephosphorylated (ERK2) forms of the kinase are indicated on the right.

Fractionation of Proteins by SDS-PAGE and Immunoblotting

Samples containing the same amounts of protein were fractionated by SDS-PAGE, according to Laemmli [19], using an 11% separating gel and a 3% stacking gel. After completion of electrophoresis, proteins were electrophoretically transferred onto a nitrocellulose membrane, and binding sites remaining on the membrane were blocked by incubation of blots for 1 hr at room temperature with 20 mM Tris–HCl, pH 7.5 at 25°, 0.15 M NaCl, and 0.05% (v/v) Tween 20 (immunoblotting buffer) containing 3% non-fat dry milk. Membranes were then incubated for 1 hr at room temperature with immunoblotting buffer containing 1% non-fat dry milk and primary antibody at a final 0.5 μg/mL concentration. After incubation, membranes were washed five times with immunoblotting buffer and were incubated for 1 hr at room temperature with peroxydase-linked anti-rabbit immunoglobulin at a 1:3000 dilution in immunoblotting buffer containing 1% non-fat dry milk. After washing, the membrane was developed by the ECL detection system. Results shown in the figures are representative of those obtained in the three or more replicate experiments performed.

RESULTS

Effect of OA and TPA on the Phosphorylation State of ERK2 in Normal Fibroblasts

The phosphorylation state of ERK2 can be easily evaluated by taking advantage of the fact that phosphorylated forms display slower mobility as compared to their dephosphorylated counterparts when analyzed by SDS-PAGE [20, 21]. In Fig. 1, we show the results obtained when extracts prepared from normal human fibroblasts were subjected to this type of analysis. As judged by detection of retarded

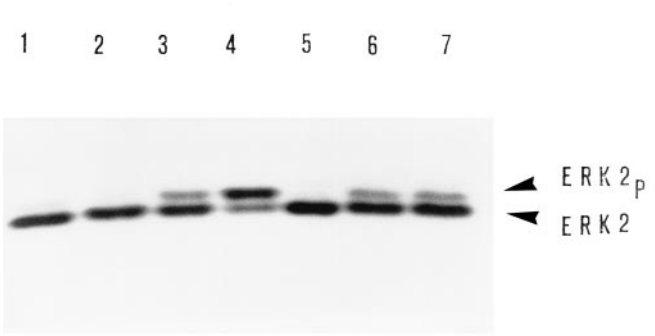

FIG. 2. Effect of OA and TPA treatment of normal mouse fibroblasts on the electrophoretic mobility of ERK2. Experimental conditions were identical to those described in the legend to Fig. 1.

bands, treatment of fibroblasts with TPA caused an immediate phosphorylation of ERK2 (Fig. 1, lane 4). This effect did not appear to be transient, as phosphorylated ERK2 remained clearly detectable after 1 hr of TPA treatment (lane 3), returning to basal levels after 1 day (lane 2). When the effect of OA was analyzed at the same time points, we found that it induced detection of higher levels of phosphorylated ERK2 as compared to control in the first hour of treatment (Fig. 1, lanes 6 and 7 vs lane 1). Treatment of normal fibroblasts for 1 day, however, resulted in decreased levels of ERK2 protein, which was detectable in its dephosphorylated state (lane 5). As the cell content of culture dishes under these experimental conditions was 70–80% of control dishes, a decreased content of ERK2 in extracts from OA-treated cells could be due to the toxic responses observable in several experimental systems after prolonged cell exposures to OA concentrations higher than 10^{-8} M [7]. The effects of tumor promoters on ERK2 were also evaluated on normal mouse fibroblasts. As is shown in Fig. 2, both TPA and OA induced an immediate/early phosphorylation of ERK2, yielding responses which reproduced those observed with fibroblasts of human origin (Fig. 1).

Effect of OA and TPA on the Phosphorylation State of ERK2 in Established Cell Lines

We next analyzed extracts prepared from established cell lines using 3T3 Swiss albino mouse fibroblasts (Fig. 3). As shown in the upper panel, TPA induced measurable phosphorylation of ERK2 within the first hour of cell treatment (lanes 3 and 4), but the effect was lost upon prolonged exposure to this agent (lane 2). If this response is compared to that found in normal fibroblasts (Figs. 1 and 2), where the vast majority of ERK2 was phosphorylated in the first minutes of response to TPA, a much weaker effect was evident in extracts prepared from phorbol ester-treated 3T3 cells (Fig. 3). Furthermore, increased levels of phosphorylated ERK2 could not be detected after OA treatment of 3T3 cells within the same time-frame employed to analyze the effects of TPA (Fig. 3, lanes 5–7). These results

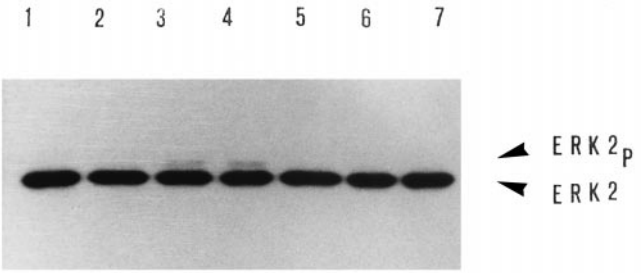

FIG. 3. Effect of OA and TPA treatment of 3T3 cells on the electrophoretic mobility of ERK2. Experimental conditions were identical to those described in the legend to Fig. 1.

prompted us to evaluate the effects of TPA and OA on a different established cell line. To this end, we chose a prostate epithelial cell line, termed PNT2, which has been immortalized by transformation of normal cells with Simian Vacuolating Virus No. 40 [15]. As shown in Fig. 4, the phosphorylation state of ERK2 was affected only if PNT2 cells received a prolonged (24-hr) treatment with OA (lane 5), whereas no measurable effect could be observed after TPA addition.

In order to gain more insight into this phenomenon, we took advantage of the fact that several strains of PNT prostate epithelial cells displaying less differentiated phenotypes have been obtained, following the order PNT2 > PNT1A > PNT1B [15]. We then treated these cells for 24 hr with 50 nM OA and analyzed the phosphorylation state of ERK2 in extracts from control and OA-treated cells, comparing the responses among the three different cell strains (Fig. 5). In agreement with the results shown in Fig. 4, OA treatment stabilized the phosphorylated forms of ERK2 in PNT2 cells (Fig. 5, compare lanes 1 and 2). The same treatment in PNT1A cells led to a much weaker stabilization of phosphorylated ERK2 as compared to controls (Fig. 5, compare lanes 3 and 4), whereas no stabilization at all could be detected in extracts prepared from PNT1B cells (lanes 5 and 6). Indeed, in this latter cell strain we consistently observed that a 24-hr treatment with OA was accompanied by a slight decrease in the levels of

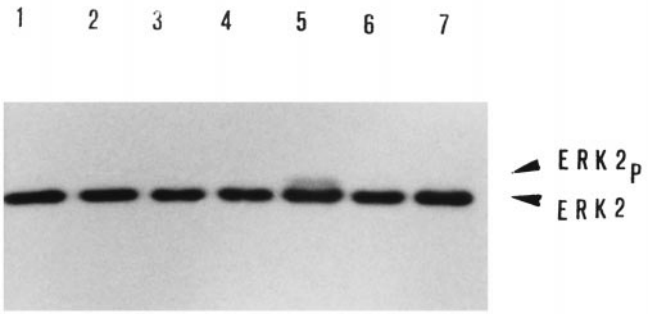

FIG. 4. Effect of OA and TPA treatment of PNT2 prostate epithelial cells on the electrophoretic mobility of ERK2. Experimental conditions were identical to those described in the legend to Fig. 1.

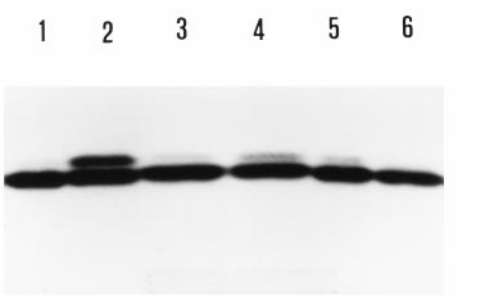

FIG. 5. Effect of OA treatment of PNT strains of prostate epithelial cells on the electrophoretic mobility of ERK2. Equal amounts of protein in extracts from control cells (lanes 1, 3, and 5) and cells which had been treated for 24 hr with 50 nM OA (lanes 2, 4, and 6) were subjected to SDS-PAGE and analyzed by immunoblotting using anti-ERK2 antibody as described under Materials and Methods. Cytosoluble extracts used in this experiment were obtained from the following cell strains: PNT2 (lanes 1, 2), PNT1A (lanes 3, 4), and PNT1B (lanes 5, 6).

phosphorylated ERK2 as compared to control (Fig. 5, compare lanes 5 and 6).

Effect of OA and TPA on the Phosphorylation State of ERK2 in Tumor Cell Lines

The limited capacity of the established cells we evaluated to respond to either TPA or OA by changing the phosphorylation states of ERK2 led us to expand our study to tumor cell lines. In Fig. 6, we report the results obtained in a typical experiment with HeLa S_3 cells, where we found that the phosphorylation state of ERK 2 was apparently unaffected by either OA or TPA under our experimental conditions. These findings were further verified using the rat HTC tumor cell line. The results obtained showed that neither the protein levels nor the phosphorylation state of ERK2 was affected by TPA and OA treatment under our experimental conditions (not shown).

DISCUSSION

The operational equivalence of TPA- and OA-type tumor promoters, as enhancers of phosphorylated states of relevant proteins, represents a good theoretical framework to explain their effects on cell proliferation [22]. We are not aware, however, of studies aimed at defining whether TPA and OA might indeed be equivalent in determining the phosphorylation state of certain proteins in different cells. The data obtained after treatment of eight different cell lines with OA or TPA show that, at least in the case of the phosphorylation states of ERK2, the responses to the two agents indeed diverged among cell lines. Enhanced phosphorylation of ERK2 in response to both OA and TPA, in fact, was detected only in normal fibroblasts, which appeared more sensitive to TPA than OA under our experimental conditions (Figs. 1 and 2). In the other cell lines tested, ERK2 phosphorylation was sensitive to either TPA (Fig. 3, 3T3 cells) or OA (Figs. 4 and 5, PNT cells) or was unaffected by both agents (Fig. 6, HeLa S_3 and HTC cells). Furthermore, when sensitivity to a single agent was detected, enhanced levels of phosphorylated ERK2 were detected within 1 hr of treatment when the effect of TPA was analyzed, whereas OA treatment could also induce late (one day) responses (Figs. 3–5).

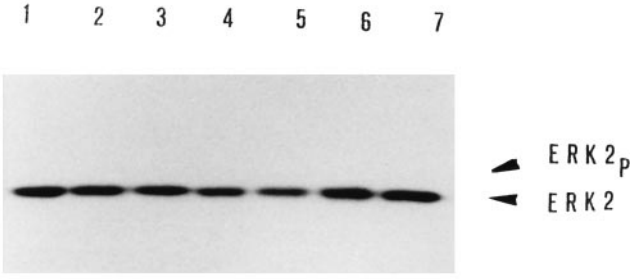

FIG. 6. Effect of OA and TPA treatment of HeLa S_3 cells on electrophoretic mobility of ERK2. Experimental conditions were identical to those described in the legend to Fig. 1.

The different kinetics of enhanced ERK2 phosphorylation detected in OA- and TPA-treated cells are in line with data obtained by others [23–26], and it seems likely that this difference depends on the molecular mechanisms underlying the actions of the two agents. TPA, in fact, would stimulate phosphate addition by activating mechanisms involving mitogen-activated/extracellular signal-regulated protein kinase [10–13], whereas OA could mimic this effect only by protecting already phosphorylated ERK2 through inhibition of PPases [3, 6–8, 11]. Alternative interpretations, however, should be considered, as enhanced MAPK phosphorylation/activation occurring within minutes of OA addition to cultured cells has been reported by others [27–29]. In those cases, high concentrations of OA (over 10^{-6} M) were used, but a faster attainement of PPase 1 and 2A inhibition under those conditions may not be sufficient to explain the effect, as we also observed an immediate/early effect when normal fibroblasts were treated with 50 nM OA (Figs. 1 and 2). Hence, different kinetics of responses in OA- and TPA-treated cells do not appear to justify rejection of the contention concerning the operational equivalence of the two classes of tumor promoters.

The operational equivalence of OA and phorbol esters regarding MAPK functioning, however, should not be generalized, as sensitivity to only one type of tumor promoter was detected with 3T3 and PNT cells (Figs. 3–5). Furthermore, in the case of immortalized prostate epithelial cells, we found that enhanced ERK2 phosphorylation due to OA was either weak or absent in some strains (Fig. 5), and a decreased sensitivity to tumor promoters was detected in immortalized mouse 3T3 fibroblasts (Fig. 3) as compared to their normal counterparts (Fig. 2). Interestingly enough, the lack of sensitivity to both OA and TPA observed with the least differentiated [15] PNT1B cell line (Fig. 5) was also detected under identical experimental conditions with HeLa S_3 (Fig. 6) and HTC (data not shown) tumor cells.

Taken as a whole, the data obtained would thus indicate that sensitivity to OA and TPA may be inversely related to the proliferative potential of cell lines, being highest in normal cells, partial in established cells, and weak or absent in tumor cells. Therefore, the proposed operational equivalence of OA- and phorbol ester-type tumor promoters would actually be maximal in the case of normal cells, which indeed may become targets susceptible of tumor promotion.

If this interpretation is correct, one implication might be that the mechanisms controlling MAPK phosphorylation/activation in tumor cells are either impaired or involve different molecular components as compared to normal cells. This latter possibility should be considered, as it is known that MAPK phosphorylation participates in different signal transduction pathways [10, 11]. Furthermore, as the full activity of MAPKs depends on the phosphorylation of both threonine and tyrosine residues [9–13], it is anticipated that cell treatment with OA would prevent the dephosphorylation of ERK2 catalyzed by PP1 and 2A [9, 30], but not other PPases [9, 14, 20, 30–34]. Although we have not directly evaluated PPases in our systems, our contention that mechanisms controlling the phosphorylation state of MAP kinases are impaired in cells displaying less differentiated and more aggressive proliferative phenotypes is supported by other experimental evidence. Leevers and Marshall [35], in fact, have shown that ERK2 is constitutively active when Swiss 3T3 cells are loaded with p21*ras* oncoprotein, and that treatment of loaded cells with TPA for 30 min does not lead to any further increase in the levels of phosphorylated kinase. Furthermore, it has been shown that the ERK activation pathway is repressed in fibroblasts transformed by *v-src*, *v-ras*, or *v-raf* [36].

On the basis of data obtained with ERK2 by us and others, an inverse relationship between tumor promoter sensitivity and transformed phenotypes may thus be proposed as a working hypothesis. A systematic evaluation of other molecular targets shared by OA- and phorbol ester-type tumor promoters will provide further insight into this issue.

This work was supported by a grant from the "Associazione Angela Serra per la Ricerca sul Cancro" (Modena, Italy) and was made possible by a kind gift from "TECNOFERRARI S.p.A." (Fiorano Modenese, Italy). We thank Dr. Philippe Berthon (Departement d'Urologie, Hôpital Saint Louis, Paris, France) for kindly providing the PNT cell lines. This study was one of the activities of our group performed within the framework of a Biomed 2 network supported by the European Union (Gene Therapy for Prostate Cancer).

References

1. Hunter T, Protein kinases and phosphatases: The yin and yang of protein phosphorylation and signalling. *Cell* **80:** 225–236, 1995.
2. Nishizuka Y, Studies and perspectives of protein kinase C. *Science* **233:** 305–312, 1986.
3. Cohen P and Cohen PTW, Protein phosphatases come of age. *J Biol Chem* **264:** 21435–21438, 1989.
4. Fujiki H and Suganuma M, Tumor promotion by inhibitors of protein phosphatases 1 and 2A: The okadaic acid class of compounds. *Adv Cancer Res* **61:** 143–194, 1993.
5. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa V and Nishizuka Y, Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* **257:** 7847–7851, 1982.
6. Bialojan C and Takai A, Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. *Biochem J* **256:** 283–290, 1988.
7. Walter G and Mumby M, Protein serine/threonine phosphatases and cell transformation. *Biochim Biophys Acta* **1155:** 207–226, 1993.
8. Wera S and Hemmings BA, Serine/threonine protein phosphatases. *Biochem J* **311:** 17–29, 1995.
9. Anderson NG, Maller JL, Tonks NK and Sturgill TW, Requirement for integration of signals from two distinct phosphorylation pathways for activation of MAP kinase. *Nature* **343:** 651–653, 1990.
10. Cobb MH and Goldsmith EJ, How MAP kinases are regulated. *J Biol Chem* **270:** 14843–14846, 1995.
11. Seger R and Krebs EG, The MAPK signaling cascade. *FASEB J* **9:** 726–735, 1995.
12. Kosako H, Gotoh Y, Matsuda S, Ishikawa M and Nishida E, *Xenopus* MAP kinase activator is a serine/threonine/tyrosine kinase activated by threonine phosphorylation. *EMBO J* **11:** 2903–2908, 1992.
13. Nakielny S, Cohen P, Wu J and Sturgill TW, MAP kinase activator from insulin-stimulated skeletal muscle is a protein threonine/tyrosine kinase. *EMBO J* **11:** 2123–2129, 1992.
14. Keyse SM, An emerging family of dual specificity MAP kinase phosphatases. *Biochim Biophys Acta* **1265:** 152–160, 1995.
15. Berthon P, Cussenot O, Hopwood L, LeDuc A and Maitland N, Functional expression of SV40 in normal human prostatic epithelial and fibroblastic cells: Differentiation pattern of non-tumorigenic cell lines. *Int J Oncol* **6:** 333–343, 1995.
16. Rossini GP and Malaguti C, Nanomolar concentrations of untransformed glucocorticoid receptor in nuclei of intact cells. *J Steroid Biochem Mol Biol* **51:** 291–298, 1994.
17. Labarca C and Paigen K, A simple, rapid, and sensitive DNA assay procedure. *Anal Biochem* **102:** 344–352, 1980.
18. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchoninic acid. *Anal Biochem* **150:** 76–85, 1985.
19. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685, 1970.
20. Sun H, Charles CH, Lau LF and Tonks NK, MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. *Cell* **75:** 487–493, 1993.
21. Rao GN, Baas AS, Glasgow WC, Eling TE, Runge MS and Alexander RW, Activation of mitogen-activated protein kinase by arachidonic acid and its metabolites in vascular smooth muscle cells. *J Biol Chem* **269:** 32586–32591, 1994.
22. Fujiki H, Suganuma M, Yoshizawa S, Nishiwaki S, Winyar B and Sugimura T, Mechanisms of action of okadaic acid class tumor promoters on mouse skin. *Environ Health Persp* **93:** 211–214, 1991.
23. Meier KE, Licciardi KA, Haystead TAJ and Krebs EG, Activation of messenger-independent protein kinases in wild-type and phorbol ester-resistant EL4 thymoma cells. *J Biol Chem* **266:** 1914–1920, 1991.
24. Amaral MC, Casillas AM and Nel AE, Contrasting effects of two tumor promoters, phorbol myristate acetate and okadaic

acid, on T-cell responses and activation of p42 MAP-kinase/ERK-2. *Immunology* **79:** 24–31, 1993.
25. Casillas AM, Amaral K, Chegini-Farahani S and Nel AE, Okadaic acid activates p42 mitogen-activated protein kinase (MAP kinase; ERK-2) in B-lymphocytes but inhibits rather than augments cellular proliferation: Contrast with phorbol 12-myristate 13-acetate. *Biochem J* **290:** 545–550, 1993.
26. Qiu Z-H and Leslie CC, Protein kinase C-dependent and -independent pathways of mitogen-activated protein kinase activation in macrophages by stimuli that activate phospholipase A_2. *J Biol Chem* **269:** 19480–19487, 1994.
27. Haystead TAJ, Weiel JE, Litchfield DW, Tsukitani Y, Fisher EH and Krebs EG, Okadaic acid mimics the action of insulin in stimulating protein kinase activity in isolated adipocytes. *J Biol Chem* **265:** 16571–16580, 1990.
28. Gotoh Y, Nishida E and Sakai H, Okadaic acid activates microtubule-associated protein kinase in quiescent fibroblastic cells. *Eur J Biochem* **193:** 671–674, 1990.
29. Miyasaka T, Miyasaka J and Saltiel AR, Okadaic acid stimulates the activity of microtubule-associated protein kinase in PC-12 pheochromocytoma cells. *Biochem Biophys Res Commun* **168:** 1237–1243, 1990.
30. Alessi DR, Gomez N, Moorehead G, Lewis T, Keyse SM and Cohen P, Inactivation of p42 MAP kinase by protein phosphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines. *Curr Biol* **5:** 283–295, 1995.
31. Gomez N and Cohen P, Dissection of the protein kinase cascade by which nerve growth factor activates MAP kinases. *Nature* **353:** 170–173, 1991.
32. Sanghera JS, McNabb CK, Tonks NK and Pelech SL, Tyrosyl phosphorylation and activation of the myelin basic protein kinase p44mpk during sea star oocyte maturation. *Biochim Biophys Acta* **1095:** 153–160, 1991.
33. Keyse SM and Emslie EA, Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. *Nature* **359:** 644–646, 1992.
34. Alessi DR, Smythe C and Keyse SM, The human CL100 gene encodes a tyr/thr-protein phosphatase which potently and specifically inactivates MAP kinase and suppresses its activation by oncogenic ras in *Xenopus* oocyte extracts. *Oncogene* **8:** 2015–2020, 1993.
35. Leevers SJ and Marshall CJ, Activation of extracellular signal-regulated kinase, ERK2, by p21*ras* oncoprotein. *EMBO J* **11:** 569–574, 1992.
36. Stofega MR, Yu C-L, Wu J and Jove R, Activation of extracellular signal-regulated kinase (ERK) by mitogenic stimuli is repressed in v-Src-transformed cells. *Cell Growth Differ* **8:** 113–119, 1997.