Volume 333, number 1,2, 114–118 FEBS 13149 October 1993

Expression of glutathione S-transferase P-form in primary cultured rat liver parenchymal cells by coplanar polychlorinated biphenyl congeners is suppressed by protein kinase inhibitors and dexamethasone

Yasunobu Aoki*, Michi Matsumoto, Kazuo T. Suzuki**

National Institute for Environmental Studies, 16–2 Onogawa, Tsukuba, Ibaraki 305, Japan

Received 18 June 1993; revised version received 6 September 1993

Glutathione S-transferase P-form (GST-P, EC 2.5.1.18) mRNA was expressed by epidermal growth factor as well as by 3,4,5,3′,4′-penta-chlorinated biphenyl (PenCB) in primary cultured rat liver parenchymal cells. The expression of GST-P was suppressed by inhibitors of protein kinase C and dexamethasone, an antagonist of AP-1 transcription factor activity, whereas expression of cytochrome P450IA2 by PenCB was not affected by these reagents. The AP-1 related transcription factor may be essential for the expression of GST-P by PenCB as also may be a protein kinase C type enzyme.

Glutathione S-transferase P-form; Coplanar polychlorinated biphenyl; Epidermal growth factor; Liver parenchymal cell; Protein kinase C; AP-1

1. INTRODUCTION

Polychlorinated biphenyl (PCB) congeners and related compounds are recognized as potent hepatocarcinogens as a result of experimental and epidemiological studies [1–4]. These congeners are also teratogenic [5] and produce various other adverse effects, such as skin lesions. Coplanar PCB congeners, which are chlorinated at the *meta* and *para* positions, have more severe effects than their non-coplanar counterparts, which are substituted at the *ortho* positions [6]. Coplanar PCB congeners are not significant mutagens and are categorized as tumor promoters [2,4]. The mechanism by which these adverse effects are induced by coplanar PCB congeners is not yet understood.

Our previous study has shown that glutathione S-transferase P-form (a placental form, GST-P, EC 2.5.1.18) was specifically induced in primary cultured rat liver parenchymal cells exposed to 3,4,5,3′,4′-pentachlorobiphenyl (PenCB), which is a most toxic coplanar congener [7]. GST-P is normally latent in the liver, but is expressed in preneoplastic hepatic foci and hepatocarcinomas [8–11].

*Corresponding author.

**Present address: Faculty of Pharmaceutical Sciences, Chiba University.

Abbreviations: EGF, epidermal growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST-P, glutathione S-transferase P-form (EC 2.5.1.18); PCB, polychlorinated biphenyl; PenCB, 3,4,5,3′,4′-penta-chlorinated biphenyl; TGF-α, transforming growth factor-α; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TRE, TPA responsive element; XRE, xenobiotic responsive element.

Epidermal growth factor (EGF) is known to induce GST-P in rat liver parenchymal cells [12]. EGF exerts its effects by activating tyrosine kinase on the EGF receptor which in turn activates protein kinases, including protein kinase C [13], through a cascade system [14,15]. Intensive studies revealed that EGF, as well as 12-*O*-tetradecanoylphorbol-13-acetate (TPA, an activator of protein kinase C), activates the transcription factor AP-1 (the heterodimer of the proto-oncogene product c-Jun and c-Fos) and a related transcription factor (e.g. the homodimer of c-Jun) via protein kinase C [15,16]. AP-1 binds to the TPA responsive element (TRE) on the flanking sequence of the structural gene resulting in its expression [15]. The region 5′-upstream of the GST-P gene was shown to contain TRE and related enhancer elements termed GPE I and II [17–20], suggesting that coplanar PCB congeners may mimic the effect of EGF, i.e. stimulation of AP-1 activity and expression of the GST-P gene.

We report here that inhibitors of protein kinase C and dexamethasone (an antagonist against AP-1 activity) suppressed expression of GST-P mRNA caused by PenCB, suggesting that an AP-1 related transcription factor may play a role in the induction of GST-P by coplanar PCB congeners in primary cultured rat liver parenchymal cells.

2. MATERIALS AND METHODS

2.1. *Materials*

PenCB and pGP5 were kindly provided by Dr. M. Morita and Drs. M. Muramatsu and M. Imagawa, respectively. pmP1450 (ATCC# 63006) was obtained from the American Tissue Culture Collection. Mouse EGF were purchased from Wako Pure Chemicals (Tokyo,

 Published by Elsevier Science Publishers B.V.

Japan); rat transforming growth factor-α (TGF-α; synthetic, residues 1–50), from Peninsula Laboratories (Belmont, USA); a cDNA probe of β-actin, from Oncor Inc. (Gaithersburg, USA); genistein and phloretin, from Extrasynthesis (Genay, France); H-7, from Seikagaku Corp. (Tokyo, Japan); TPA, from LC Service (Woburin, USA) and dexamethasone, from Sigma (St. Louis, USA).

2.2 *Preparation of monolayer cultured rat liver parenchymal cells and their exposure to chemicals*

Preparation of cultured cells and their chemical exposure were essentially performed as previously reported [7]. Liver parenchymal cells were isolated from a rat liver digested with a collagenase solution, and 0.85×10^6 cells were plated in collagen-coated 6-well (diameter 35 mm) plates with 1.5 ml of culture medium (William's E medium (Sigma) containing 10 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml fungizon and 25 mM HEPES/NaOH buffer, pH 7.4) containing 1 nM insulin, 100 nM dexamethasone and 10% (v/v) fetal calf serum, and were cultured for 4 h in a humidified incubator at 37°C under 95% air/5% CO_2. The next medium used was serum-free culture medium containing 1 nM insulin, 100 nM dexamethasone and 0.1 μg/ml aprotinin. Following further incubation for 18 h, the cells were treated with either PenCB or EGF for 12 h or for the time indicated with a change in culture medium to one containing 6 mM nicotinamide, 0.1 μg/ml aprotinin as well as the other reagents at the indicated concentrations. The chemicals and EGF were dissolved in dimethyl sulfoxide and 1% bovine serum albumin, respectively, to make stock solutions at 1,000-fold concentrations and these were added to the culture medium immediately before use.

2.3. *Extraction of RNA and northern blot analysis of mRNA*

Total RNA was extracted from the cells as described by Chomczynski and Sacchi [21]. RNA (20 μg) was separated by electrophoresis on a formaldehyde/1.2% agarose gel. The separated RNA was transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, USA) or a nylon membrane (Amersham, Buckinghamshire, UK) and immobilized [22,23]. The membranes were hybridized with c-DNA probes of GST-P [24] and cytochrome P450IA2 [25], which are an *Eco*RI–*Sal*I fragment of pGP5 [26] and a *Pst*I fragment of pmP1450 [27], respectively, after labeling with [^{32}P]dCTP (Amersham) using a random primed DNA labeling kit (Boehringer, Mannheim, Germany). cDNA probes of β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as controls [22]. A cDNA probe of GAPDH (the 855 bp fragment corresponding to exons sandwitched with a portion of the third exon and the ninth exon [28]) was kindly provided by Drs. T. Fujii and Y. Mitsui. The hybridized cDNA was detected by autoradiography.

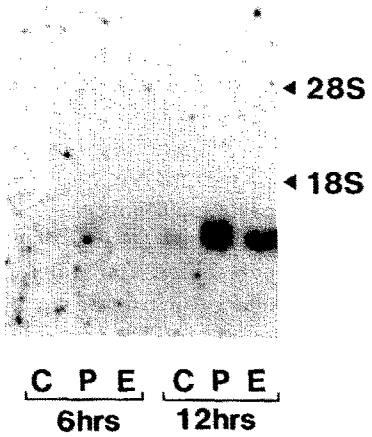

Fig. 1. Expression of GST-P mRNA by PenCB and EGF. Primary cultured rat liver parenchymal cells were incubated without pretreatment (C) or in the presence of 100 nM PenCB (P) or 10 ng/ml EGF (E) for 6 h and 12 h. After total RNA was extracted from the cells, GST-P mRNA levels were determined by Northern blotting analysis. 28S and 18S indicate the positions of 28S and 18S RNA, respectively.

3. RESULTS

Primary cultured rat liver parenchymal cells were incubated with either 100 nM PenCB or 10 ng/ml EGF, and the amount of GST-P mRNA was determined. PenCB and EGF were both effective inducers of GST-P, and comparable amounts of GST-P mRNA were expressed after treatment with EGF or PenCB for 12 h (Fig. 1). TGF-α, which shares the same receptor with EGF on the plasma membrane, also induced GST-P (data not shown).

PenCB appeared to act on the EGF receptor. To verify this, the cells were exposed to PenCB in the presence of genistein (a tyrosine kinase inhibitor) [29]. As shown in Fig. 2a, genistein had no inhibitory effect on

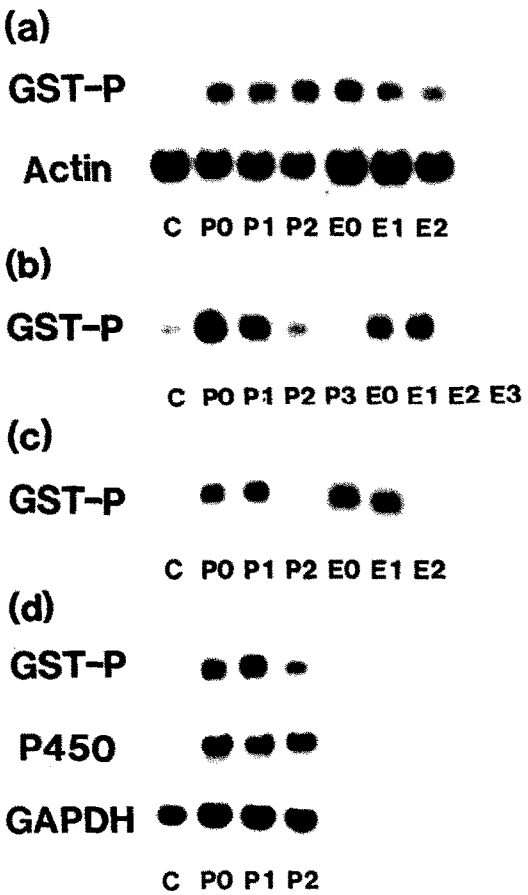

Fig. 2. Effect of protein kinase inhibitors on levels of GST-P and other mRNAs. Cells were incubated with 100 nM PenCB (lanes P0–P3) or 10 ng/ml EGF (E0–E3) for 12 h in the absence (P0 and E0) or presence of protein kinase inhibitors (P1–P3 and E1–E3). (a) The level of GST-P mRNA was determined after treatment with EGF and PenCB in the presence of genistein (P1 and E1, 4 μM; P2 and E2, 20 μM). The level of β-actin mRNA was also determined (Actin). (b) H-7 (P1 and E1, 5 μM; P2 and E2, 25 μM; P3 and E3, 100μM). (c) phloretin (P1 and E1, 20 μM; P2 and E2, 100 μM). (d) Effect of H-7 on levels of GST-P, cytochrome P450IA2 (P450) and GAPDH mRNA (P1 and E1, 5 μM; P2 and E2, 25 μM). C indicates mRNA levels in the control cells.

expression of GST-P mRNA stimulated by PenCB, although the expression by EGF was reduced. The amount of β-actin mRNA was basically similar for all RNA preparations. This suggests that the tyrosine kinase activity of the EGF receptor is not directly related to the expression of the GST-P gene by PenCB.

Various protein kinases, including protein kinase C, are known to be activated by tyrosine kinase on the EGF receptor through the cascade system [16]. One of these protein kinases may participate in GST-P expression by PenCB. Liver parenchymal cells were treated with protein kinase inhibitors in the presence of either PenCB or EGF. H-7 (a synthetic inhibitor of protein kinase C and cyclic nucleotide dependent protein kinase) [30] reduced expression of GST-P mRNA by both EGF and PenCB to that of the control level (25 μM), which is close to the inhibition constant of H-7 for protein kinase C activity (6 μM) (Fig. 2b). GST-P expression by PenCB was also inhibited by treatment with phloretin, an inhibitor of protein kinase C [31] (Fig. 2c). Cytochromes P450IA1 and P450IA2 are typical gene products specifically expressed by coplanar PCB congeners and related compounds [5] and cytochrome P450IA2 is a major iso-protein induced in primary cultured rat liver parenchymal cells [26,32]. We examined whether expression of cytochrome P450IA2, as well as GST-P, by PenCB was sensitive to H-7. As shown in Fig. 2d, the expression of cytochrome P450IA2 mRNA was not inhibited by H-7 at the concentration at which GST-P expression was suppressed. Levels of GAPDH mRNA were similar among RNA preparations. Expression of cytochrome P450IA2 was shown to be unaffected by H-7.

Fig. 3 shows that GST-P mRNA was detected after the liver parenchymal cells were incubated with 10 ng/ml TPA instead of PenCB, however, the level of GST-P mRNA induced by TPA was less than that in the PenCB treated cells. Expression of GST-P mRNA by TPA was also inhibited by H-7 at the same concentration at which GST-P expression by PenCB was inhibited (data not shown). Dibutyryl cyclic AMP (2 mM), an activator of protein kinase A, did not affect expression of GST-P mRNA. These results suggest that protein kinase C or a related protein kinase plays a role in the induction of GST-P in liver parenchymal cells.

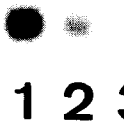

Fig. 3. Effect of protein kinase activators on levels of GST-P mRNA. After the cells were treated with 100 nM PenCB (lane 1), 10 ng/ml TPA (lane 2) or 2 mM dibutyryl cyclic AMP (lane 3) for 12 h, the GST-P mRNA level was determined. C is the level of GST-P mRNA in the control cells.

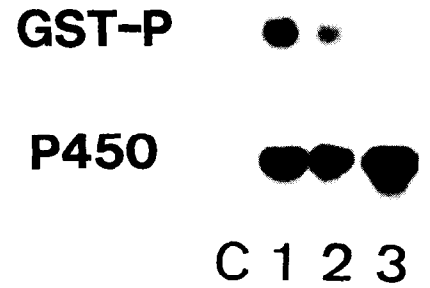

Fig. 4. Suppression of GST-P mRNA by dexamethasone. After the cells were exposed to 100 nM PenCB in the absence (lane 1) or presence of dexamethasone (lane 2, 10 nM; lane 3, 100 nM) for 12 h, levels of GST-P and cytochrome P450IA2 (P450) mRNA were determined. C is the mRNA level in the control cells.

TRE-containing genes are activated by the binding of AP-1 to TRE, and the binding ability of AP-1 is regulated by protein kinase C through the protein kinase cascade system [16]. If AP-1 is required for the induction of GST-P by PenCB, this induction could be suppressed by dexamethasone which acts as an antagonist against AP-1 activity by means of a mechanism by which the dexamethasone-glucocorticoid receptor complex is bound to AP-1, abolishing its capacity to bind to TRE [35–37]. Dexamethasone has been previously reported to reduce spontaneous induction of GST-P in extended cultures of hepatocytes [38]. As expected, after the cells were exposed to PenCB in the presence of 100 nM dexamethasone, the expression of GST-P mRNA was completely suppressed, dropping to the level of non-exposed cells (Fig. 4). The expression of cytochrome P450IA2 mRNA, however, was not affected by dexamethasone.

4. DISCUSSION

GST-P was shown to be induced by EGF as well as by PenCB. Although GST-P expression by PenCB was not affected by a tyrosine kinase inhibitor, expression by both PenCB and EGF was inhibited by protein kinase C inhibitors. The present study also demonstrates that expression of GST-P mRNA by PenCB was suppressed by dexamethasone, an antagonist against AP-1 activity. These results suggest that AP-1 or a related transcription factor is essential for expression of GST-P by coplanar PCB congeners in primary cultured rat liver parenchymal cells, as is a protein kinase C type enzyme.

Coplanar PCB congeners are well-documented inducers of cytochromes P450IA1 and IA2 and several drug metabolizing enzymes [31,34]. Gene expression of these enzymes is dependent on an intracellular Ah receptor and auxiliary factors (Ah receptor system) and on 5′-flanking regions of these genes which contain an Ah receptor binding site termed the xenobiotic responsive element (XRE) [34]. Although XRE is not found on

the 5'-flanking sequence of GST-P, TRE and related responsive elements (GPE I and II) are contained in this sequence, and these responsive elements are known to be essential for the expression of GST-P in carcinoma cells [19,20]. In this study, expression of cytochrome P450IA2 gene (a typical XRE containing gene) by PenCB was not affected by kinase C inhibitors or by dexamethasone, although expression of GST-P was suppressed by these reagents, suggesting that the induction of GST-P by coplanar PCB congeners is likely to be controlled by a novel type of regulatory system other than the Ah receptor system [39].

The current consensus of how TRE-containing genes are expressed is that TPA activates protein kinase C and this enzyme regulates activity of AP-1 causing it to bind to TRE via phosphorylation through the protein kinase cascade [15,40]. Expression of GST-P mRNA by TPA in primary cultured liver parenchymal cells indicates that TPA activates AP-1 or related transcription factors in this type of cell. Dexamethasone suppressed the expression of GST-P, suggesting that AP-1 or a related transcription factor plays a role in GST-P expression by coplanar PCB congeners. C-Jun mRNA was reported to be endogenously expressed in primary cultured liver parenchymal cells, but there was only a slight expression of c-Fos mRNA [24]. We observed that expression of these mRNAs was not affected by PenCB (data not shown). A homodimer of c-Jun may act as an AP-1 transcription factor in the cells.

If AP-1 activity is essential for the expression of GST-P by PenCB in these cells, coplanar PCB congeners, as well as TPA, may activate protein kinase C or a related protein kinase, resulting in activation of AP-1. Coplanar PCB congener-dependent protein kinase activity has not been identified, but several observations regarding this activity have been reported [41,42]. Several factors other than a protein kinase might influence gene expression of GST-P by coplanar PCB congeners, since the magnitude of expression by PenCB was much higher than by TPA. Further studies on GST-P expression by PenCB in primary cultured liver parenchymal cells are important for understanding the various effects of coplanar PCB congeners.

Acknowledgements: We wish to thank Dr. M. Morita (NIES), Drs. M. Muramatsu (Saitama Medical College), M. Imagawa (Osaka Univ.) and Drs. T. Fujii and Y. Mitsui (National Institute of Bioscience and Human Technology) for kindly donating PenCB, pGP5 and a cDNA probe of GAPDH, respectively. We also are grateful to Drs. I. Hatayama and K. Satoh (Hirosaki Univ.) for their critical discussions and Drs. T. Suzuki and T. Miura (NIES) for their encouragement.

REFERENCES

[1] IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 18 (1978) IARC/WHO, Lyon.
[2] Pitot, H.C., Goldsworthy, T., Cambell, H.A. and Poland, A. (1980) Cancer Res. 40, 3616–3620.
[3] Kuratsune, M., Nakamura, Y., Ikeda, M. and Hirihata, T. (1987) Chemosphere 16, 2085–2088.
[4] Birnbaum, L.S. (1991) in: Biological Basis for Risk Assessment of Dioxins and Related Compounds (Gallo, M.A., Scheuplein, R.J. and van der Heijden, K.A., Eds.) Banbury Report Vol. 35, pp. 51–67, Cold Spring Harbor Laboratory Press, New York.
[5] Markes, T.A., Kimmel, G.L. and Staples, R.E. (1981) Toxicol. Appl. Pharmacol. 61, 269–276.
[6] Parkinson, A., Safe, S.H., Robertson, L.W., Thomas, P.E., Ryan, D.E., Reik, L.M. and Levin, W. (1983) J. Biol. Chem. 258, 5967–5976.
[7] Aoki, Y., Satoh, K., Sato, K. and Suzuki, K.T. (1992) Biochem. J. 281, 539–543.
[8] Sato, K., Kitahara, A., Satoh, K., Ishikawa, T., Tatematsu, M. and Ito, N. (1984) Jpn. J. Cancer Res. 75, 199–202.
[9] Satoh, K., Kitahara, A., Soma, Y., Inaba, Y., Hatayama, I. and Sato, K. (1985) Proc. Natl. Acad. Sci. USA 82, 3964–3968.
[10] Sugioka, Y., Fujii-Kuriyama, Y., Kitagawa, T. and Muramatsu, M. (1985) Cancer Res. 45, 365–378.
[11] Sato, K. (1989) Adv. Cancer Res. 52, 205–255.
[12] Hatayama, I., Yamada, Y., Tanaka, K., Ichihara, A. and Sato, K. (1991) Jpn. J. Cancer Res. 82, 807–814.
[13] Nishizuka, Y. (1986) Science 233, 305–312.
[14] Yarden, Y. and Ullrich, A. (1988) Annu. Rev. Biochem. 57, 443–478.
[15] Cantley, L.C., Auger, K.R., Carpenter, C., Duckworth, B. Graziani, A., Kapeller, R. and Soltoff, S. (1991) Cell 64, 281–302.
[16] Quantin, B. and Breathnach, R. (1988) Nature 334, 538–539.
[17] Sakai, M., Okuda, A. and Muramatsu, M. (1988) Proc. Natl. Acad. Sci. USA 85, 9496–9460.
[18] Okuda, A., Imagawa, M., Maeda, Y., Sakai, M. and Muramatsu, M. (1989) J. Biol. Chem. 264, 16919–16926.
[19] Okuda, A., Imagawa, M., Sakai, M. and Muramatsu, M. (1990) EMBO J. 9, 1131–1135.
[20] Sakai, M., Muramatsu, M. and Nishi, S. (1992) Biochem. Biophys. Res. Commun. 187, 976–983.
[21] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[22] Saida, K., Mitsui, Y. and Ishida, N. (1989) J. Biol. Chem. 264, 14613–14616.
[23] Kunimoto, M., Otto, E. and Bennett, V. (1991) J. Cell Biol. 115, 1319–1331.
[24] Morimura, S., Okuda, A., Sakai, M., Imagawa, M. and Muramatsu, M. (1992) Cell Growth Differ. 3, 685–691.
[25] Silver, G. and Krauter, K.S. (1988) J. Biol. Chem. 263, 11802–11807.
[26] Suguoka, Y., Kano, T., Okuda, A., Sakai, M., Kitagawa, T. and Muramatsu, M. (1985) Nucleic Acids Res. 13, 6049–6057.
[27] Kimura, S., Gonzalez, F.J. and Nebert, D.W. (1984) J. Biol. Chem. 259, 10705–10703.
[28] Tso, J.Y., Sun, X.H., Kao, T., Reece, K.S. and Wu, R. (1985) Nucleic Acid. Res. 13, 2485–2502.
[29] Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. and Fukami, Y. (1987) J. Biol. Chem. 262, 5592–5595.
[30] Hidaka, H., Inagaki, M., Kawamoto, S. and Sasaki, Y. (1984) Biochemistry 23, 5036–5041.
[31] Gschwendt, M., Horn, F., Kittstein, W., Furstenberger, G., Besemfelder, E. and Marks, F. (1984) Biochem. Biophys. Res. Commun. 124, 63–68.
[32] Aoki, Y., Silbergeld, E.K., Max, S.R. and Fowler, B.A. (1991) Biochem. Pharmacol. 42, 1195–1201.
[33] Nebert, D.W, and Gonzalez, F.J. (1987) Annu. Rev. Biochem. 56, 945–993.
[34] Yao, E.F. and Denison, M.S. (1992) Biochemistry 31, 5060–5067.
[35] Jonat, C., Rahmsdorf, H.J., Park, K., Cato, A.C.B., Gebel, S., Ponta, H.and Herrlich, P. (1990) Cell 62, 1189–1204.
[36] Yang-Yen, H., Chambard, J., Sun, Y., Smeal, T., Schmidt, T.J., Drouin, J. and Karin, M. (1990) Cell 62, 1205–1215.

[37] Schule, R., Rangarajan, P., Kliewer, S., Ransone, L.J., Bolado, J., Yang, N., Verma, I.M. and Evans, R.M. (1990) Cell 62, 1217–1226.
[38] Abramovitz, M., Ishigaki, S. and Listowsky, I. (1989) Hepatology 9, 235–239.
[39] Pickett, C.B. and Lu, A.Y.H. (1989) Annu. Rev. Biochem. 58, 743–764.
[40] Boyle, W.J., Smeal, T., Defize, L.H.K., Angel, P., Woodgett, J.R., Karin, M. and Hunter, T. (1991) Cell 64, 573–584.
[41] Bombick, D.W., Jankun, J., Tullis, K. and Matsumura, F. (1988) Proc. Natl. Acad. Sci. USA 85, 4128–4132.
[42] Moser, G.J. and Smart, R.C. (1989) Carcingenesis 10, 851–856.