Increased Choline Kinase Activity in 1,2-Dimethylhydrazine-induced Rat Colon Cancer

Katsunao Nakagami,[1] Tsutomu Uchida,[2] Susumu Ohwada,[1, 3] Yukio Koibuchi[1] and Yasuo Morishita[1]

[1]Second Department of Surgery and [2]Department of Biochemistry, Gunma University School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511

Cancer cells acquire particular characteristics that benefit their proliferation. We previously reported that human colon cancers examined had increased choline kinase activity and phosphocholine levels. The elevated phosphocholine levels were in part due to both activation of choline kinase and increased choline kinase α protein levels. In this report, we analyzed choline kinase, which catalyzes the phosphorylation of choline to produce phosphocholine, in rat 1,2-dimethylhydrazine (DMH)-induced colon cancer. This study is the first to demonstrate increased choline kinase α enzymatic activity, protein levels, and mRNA levels in DMH-induced colon cancer as well as human colon cancer, although phosphocholine was not increased in DMH-induced rat cancer. The increase in the mRNA level was partly due to an increase in the transcription of the choline kinase α gene. The increased choline kinase activity may be a specific characteristic acquired by cancer cells that benefits their proliferation.

Key words: Choline kinase — DMH — Cancer

Cancer of the colon is one of the most common cancers in developed countries and its prevention is of great interest throughout the world. It is thought that an accumulation of mutated genes, including oncogenes, tumor suppressor genes, DNA-repair enzyme genes, and invasion/metastasis-related genes, is necessary for the generation and progression of cancer. Mutation may cause further malignant changes in cellular proliferation,[1] especially when enzymatic activity and properties are affected.[2–4] Some of the changes that occur in enzymatic properties and activity with proliferation may favor the growth of cancer cells.[5–7] Studying the cellular properties of cancer cells furthers our understanding of the mechanisms of cellular growth control and provides clues to strategies for cancer prevention and treatment.[8]

1,2-Dimethylhydrazine (DMH) is widely used for experimental studies of specific colon carcinogenesis in rodents.[9] In the body, the metabolic product of DMH modifies DNA, causes mutation, and leads to carcinogenesis.[10]

Choline kinase is the first enzyme in the CDP-choline pathway for the synthesis of phosphatidylcholine, and phosphorylates choline to phosphocholine using adenosine 5′-triphosphate (ATP) as the phosphate donor.[11, 12] *Ras* proteins play a pivotal role in cellular signal transduction, and help regulate cellular proliferation and terminal differentiation.[13–15] Microinjection of the oncogenic Ha-*ras* gene product p21^{ras} into *Xenopus* oocytes, which causes

meiosis,[16] quickly elevates the phosphocholine level and activates choline kinase.[17] Transformation of fibroblastic cells with oncogenic Ha-*ras* activates choline kinase.[18–20] Growth factors essential for cellular growth also activate choline kinase, elevating the intracellular phosphocholine level. It has been suggested that platelet-derived growth factor might use a choline kinase-phosphocholine route to promote cell growth in NIH3T3 fibroblast cells.[21–24] We previously reported that human colon cancers examined had increased choline kinase activity and phosphocholine levels. The elevated phosphocholine levels were due in part to the activation of choline kinase and the increased choline kinase α protein levels.[25] These results suggest that choline kinase and phosphocholine may play a role not only in phospholipid synthesis, but also in regulating cellular growth in cancer cells.

This study is the first to analyze choline kinase in DMH-induced rat colon cancer. We further analyzed the mechanism of activation and found that the activation involved increases in both the protein and mRNA levels. Transcription occurred at a specific transcription start site, which is not used in the liver or testis of normal adult rats, but is used in the liver of rats treated with 3-methylcholanthrene or carbon tetrachloride.[26, 27] The particular transcription factor or pathway that stimulates this choline kinase α gene factor may be activated in DMH-induced colon cancer.

MATERIALS AND METHODS

Materials   Choline oxidase, horseradish peroxidase, bovine intestine alkaline phosphatase, and 4-aminoantipy-

---

rine were obtained from Wako Chemicals (Tokyo). DMH was purchased from Nacalai Tesque (Kyoto). The ECL western blotting detection reagent was purchased from Amersham International (Buckinghamshire, UK). Goat anti-rabbit IgG antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). [Methyl-^{14}C]choline chloride (54 mCi/mmol), [α-^{32}P]deoxycytidine 5′-triphosphate (3000 Ci/mmol), and [α-^{32}P]uridine 5′-triphosphate (800 Ci/mmol) were purchased from Du Pont New England Nuclear (Boston, MA). Glutathione Sepharose and formyl-Cellulofine were obtained from Pharmacia Biotech (Uppsala, Sweden) and Seikagaku Kogyo (Tokyo), respectively.

Animal and tissue treatment Male Wistar rats were obtained from the Institute of Experimental Animal Research, Gunma University School of Medicine, and housed under standard conditions with a 12-h light/12-h dark cycle and free access to rat chow and water. Ten-week-old rats were administered DMH dissolved in 1 mM EDTA, pH 6.5, subcutaneously in the right thigh at a dose of 20 mg/kg body weight once a week for 16 weeks.[28] Control rats were treated with a saline-EDTA solution. Then the rats were kept without any treatment for an additional 10 weeks. The rats were starved for 12 h before being killed. The large intestine was resected and washed with cold buffer (20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 5 mM 2-mercaptoethanol and 0.2 mM phenylmethylsulfonyl fluoride) containing 100 mM NaCl. Tumor and normal tissues were harvested, frozen in liquid nitrogen, and then stored at −80°C.

Preparation of cytosol Tissues were homogenized on ice in three volumes of homogenizing buffer (250 mM sucrose, 10 mM Tris-HCl (pH 7.5), 5 mM EDTA, 50 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, and 2 μg/ml leupeptin). The homogenates were centrifuged at 4°C for 10 min at 800g and then for 1 h at 100,000g. The supernatants were used as enzyme sources.[29]

Choline kinase assay Choline kinase activity was measured isotopically with [methyl-^{14}C]choline as described previously.[30]

Determination of choline and phosphocholine Choline and phosphocholine were measured spectrophotometrically using a combination of alkaline phosphatase and choline oxidase as described previously.[25]

Affinity purification of anti-choline kinase α antibody Anti-choline kinase α antibody was purified as described previously.[25, 31]

Western blot analysis The cytosolic proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis,[32] and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore Corp., Bedford, MA). Choline kinase α was detected with the affinity-purified anti-choline kinase α antibody and goat anti-rabbit IgG using the ECL reagent as reported previously.[26]

Preparation of total RNA RNA was extracted by the acid guanidine isothiocyanate phenol method,[33] and further purified by ultracentrifugation.[34] The RNA thus purified was used as total RNA in the experiments.

Northern blot analysis Total RNA was fractionated by electrophoresis in 1.2% agarose containing formaldehyde, and transferred onto nylon membranes (BioDyne A; Pall Biosupport, Glen Cove, NY). Hybridization was carried out with rat choline kinase α1 cDNA labelled by a random priming method with [α-^{32}P]deoxycytidine 5′-triphosphate.[35] Choline kinase α mRNA was detected using a Bio-Imaging Analyser BAS2000 (Fuji Photo Film Co., Ltd., Minamiashigara).

Ribonuclease protection assay Ribonuclease protection assays were carried out to examine spliced transcripts of choline kinase α and transcription start sites.[27] Antisense RNA probes labelled with [α-^{32}P]uridine 5′-triphosphate were prepared by subcloning the *Eco*81I/*Bss*HII (positions −220 to +47) fragment into pBluescript II SK+ at the *Sma*I site to determine the transcription start site, and by subcloning the *Eco*RI/*Bam*HI (positions 237 to 429) fragment from choline kinase α2 cDNA into pBluescript II SK+ between the *Eco*RI and *Bam*HI sites to analyze isoform expression. Protected fragments were separated in polyacrylamide gels and exposed to X-ray films and phospho imaging plates.

Protein assays Protein concentrations were determined using BioRad protein assay dye reagent with bovine serum albumin as the reference standard.[36]

Statistical analyses The results are expressed as the mean±the standard error of the mean (SEM). The statistical significance of differences between groups were examined with the Kruskal-Wallis test and Student's *t*-test. A P value less than 0.05 was considered to be significant.

Ethics This study conformed to the ethical guidelines of Gunma University School of Medicine and the UKCCCR 'Guidelines for the Welfare of Animals in Experimental Neoplasia'[37] were helpful in this regard.

RESULTS

General observations The control rats appeared healthy and bore no cancers. On the other hand, all the DMH-treated rats had colon cancers without metastasis on both macro- and microscopic examination. In this report, cancerous tissues were compared with those of age-matched control rats.

Choline kinase activity increased by carcinogenesis The cancer tissues studied in this report were typically around 10 mm in diameter. The choline kinase activity was compared in control normal colon, non-tumor colon of cancer-bearing rats, and colon cancer tissues (Fig. 1). Cancer tissues, 0.97±0.072 nmol/min/mg (mean±SE; n= 6), had almost twice the activity of the normal colon of
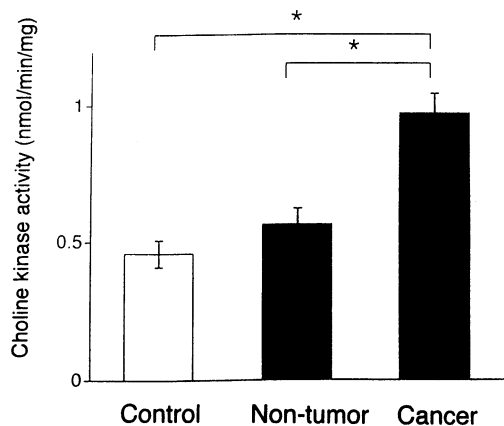
Fig. 1. Choline kinase activity. The enzyme activity of the cytosol from the normal colon of control rats (Control), normal colon of DMH-treated rats (Non-tumor) and colon cancer tissues of DMH-treated rats (Cancer) ($n=6$). ∗: $P<0.01$, control rat normal colon vs. cancer tissues; $P<0.01$, cancer-bearing rat normal colon vs. cancer tissues.

Fig. 2. Western blot analysis of choline kinase α. Cytosol (50 μg protein/lane) from the normal colon of control rats (Control), colon cancer tissues of DMH-treated rats (Cancer), and normal colon of DMH-treated rats (Non-tumor) was subjected to SDS-polyacrylamide gel electrophoresis, and then transferred onto a nylon membrane. Choline kinase α was detected with affinity-purified anti-choline kinase $\alpha 1$ antibody using ECL reagent. The levels of the 52 kDa and 50 kDa choline kinase α proteins were increased in cancer tissues.

Fig. 3. Northern blot analysis of choline kinase α. Total RNA was subjected to agarose gel electrophoresis and transferred onto a nylon membrane. Choline kinase α messages were detected with ^{32}P-labelled choline kinase $\alpha 1$ cDNA using a BAS2000. The 2.8 kb and 1.8 kb choline kinase α mRNA levels[26] were increased in cancer tissues. We used Perfect RNA Markers, 0.36–9.5 kb (Novagen Inc., Madison, WI) as molecular size markers.

controls and non-tumorous colon of cancer-bearing rats, 0.46±0.048, and 0.57±0.059, respectively.

The addition of cytosol from cancerous tissue to normal cytosol only resulted in an additive increase in the choline kinase activity. Therefore, the increased choline kinase activity was not due to an increase in intracellular choline kinase activators.

Choline and phosphocholine levels The elevation of choline kinase activity in cancer tissues should lead to an increase in the phosphocholine level. Therefore, the concentrations of phosphocholine were determined along with those of choline. There were no differences in the choline and phosphocholine levels between the cancer tissues, control normal colon, and non-tumor colon of cancer-bearing rats.

Increase in the choline kinase α protein level In order to examine the mechanism of the increased choline kinase activity in cancer tissues, we conducted a western blot analysis. The result shows that the increased activity was in part due to an increase in the choline kinase α protein level (Fig. 2).

Increase in the choline kinase α mRNA level with carcinogenesis In order to examine whether the increase in the choline kinase α protein level is due to an increase in the choline kinase α mRNA level, we next carried out a northern blot analysis. Fig. 3 shows that there was a higher level of choline kinase α transcripts in cancer tissues. This increase in mRNA was further confirmed by means of a ribonuclease protection assay (Fig. 4).

A previous report[27] found that choline kinase α isoforms $\alpha 1$ and $\alpha 2$ were derived by alternative splicing in
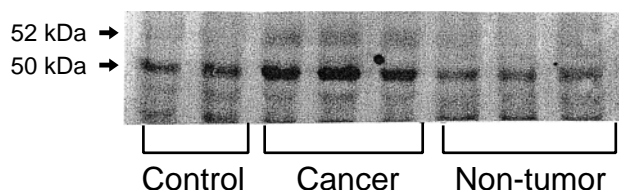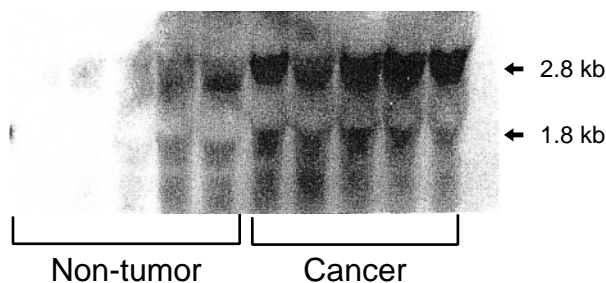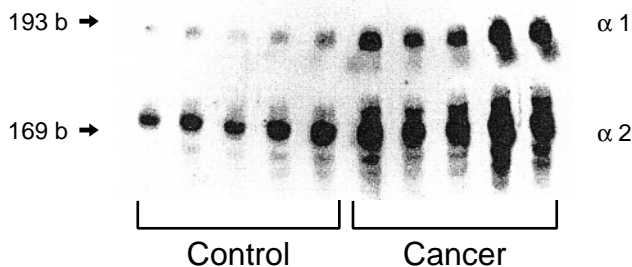

Fig. 4. Ribonuclease protection assay for transcripts of choline kinase α spliced isoforms. Total RNA (20 μg of each sample) was annealed with the ^{32}P-RNA probe, digested with RNases T1/A, and then quantitatively analyzed by polyacrylamide gel electrophoresis. The gel was exposed to X-ray film. In cancer tissues, the two spliced isoforms (193 b and 169 b) were significantly increased. Control: protected fragments from normal colon of control rats. Cancer: protected fragments from colon cancer tissues.
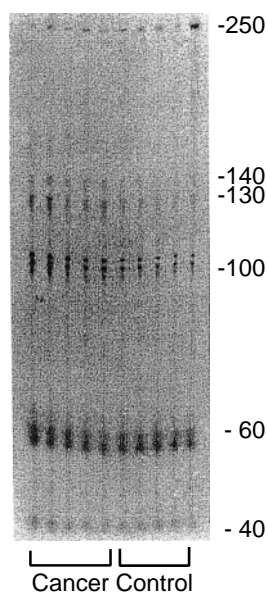
Fig. 5. Ribonuclease protection analysis for the transcription start sites of the choline kinase α gene. Total RNA (20 μg of each sample) was annealed with a ^{32}P-RNA probe, digested with RNases T1/A, and then analyzed by polyacrylamide gel electrophoresis. The gel was exposed to an X-ray film and the protected fragments were quantitatively analyzed with a Phospho Imager. In cancer, the transcription start sites were increased in positions 100–140. Control: protected fragments from the normal colon of control rats. Cancer: protected fragments from colon cancer tissue.

rat tissues. So, we examined whether DMH-induced cancer altered the splicing pattern. Both the α1 and α2 isoforms significantly increased in the cancer tissues without a change in the proportion of the two forms (Fig. 4).

Elevation of transcription with carcinogenesis The increase in the choline kinase α transcript level with carcinogenesis suggests stabilization of the message, and/or elevated transcription. Since the measurement of mRNA stabilization in an animal's body is very difficult, we analyzed whether carcinogenesis changed the transcription properties. Since several transcription start sites for choline kinase α isoforms exist and are regulated differently,[27] an RNase protection analysis, which compares the start sites quantitatively, was carried out. The transcription from start sites in positions 100–140 significantly increased (Fig. 5), which may suggest that transcription of the choline kinase α gene increases in cancer.

DISCUSSION

Choline kinase may favor the generation and progression of cancer as well as the accumulation of certain genetic mutations. The phospholipid serves as a precursor for the synthesis of other phospholipids, phosphatidylserine,[38] and sphingomyelin.[39] These phospholipids are the major building blocks of biological membranes and precursors for signal transduction.[8, 40] Choline kinase activity is increased in human colon[25] and gastric cancer (Nakagami *et al.*, unpublished data). The increased choline kinase activity may be necessary for building membranes along with cell growth and proliferation, and for rebuilding phospholipids after they are lost due to degradation when used for signal transduction.

In our study, choline kinase activity was increased in cancer tissues, and both protein and mRNA levels were increased. Two spliced forms of choline kinase, α1 and α2, are known. In most tissues, the choline kinase α2 isoform is expressed more strongly than the choline kinase α1 isoform, whereas in the testis, where new cells are constantly generated, the choline kinase α1 isoform is expressed more strongly than the choline kinase α2 isoform.[27] So, we examined whether DMH-induced cancer has an altered pattern of spliced isoforms. The two spliced forms of choline kinase, α1 and α2, however, were equally increased in DMH-induced rat cancer.

We previously reported that both choline kinase activity and phosphocholine levels are increased in human colon cancers.[25] However, choline kinase activity alone increased while phosphocholine did not in DMH-induced rat cancer. Increased choline kinase activity should increase phosphocholine levels in DMH-induced rat cancer as well as human colon cancers, and it is not clear why this is not the case. Possible explanations include differences in phosphocholine-metabolizing enzymes, such as phosphocholine cytidylyltransferase or alkaline phosphatase,[11, 12, 41] in human and DMH-induced colon cancers, or differences in other characteristics of the two cancers.

The increased choline kinase α mRNA level was in part due to elevated transcription. Transcription was accelerated from a start site that is not used in the liver or testis of normal adult rats, but becomes active after the administration of 3-methylcholanthrene or carbon tetrachloride.[27] The factors that stimulate transcription from this site or the signal transduction pathway for these transcription factors may be activated in DMH-induced colon cancer, leading to activation of the choline kinase α gene. Transcription from this start site in normal colon mucosa suggests that the transcription start site is used specifically in this tissue for basal level expression, or becomes active as the result of the constant exposure of colonic epithelial cells to xenobiotics and bile salts. Xenobiotics may act on the colon mucosa in a manner similar to the way that 3-methylcholanthrene and carbon tetrachloride act on the rat liver. Bile salts are promoters of cancer of the large intestine.[42]

The transcription from this site is controlled through some *cis*-acting element. Sequences similar to the xenobi-

otic responsive element (XRE)[5, 43] and the antioxidant responsive element (ARE) core sequences[44] are located nearby. The XRE was identified in the cytochrome P-450 gene,[43, 45] which regulates phase I metabolizing enzymes,[6, 7] and the ARE is contained in NAD(P)H: quinone reductase and glutathione *S*-transferase *Ya* subunit genes, whose transcription is activated by phenolic antioxidants and metabolizable planar aromatic compounds. Increased expression of the choline kinase α gene is induced by 3-methylcholanthrene in rat liver.[26, 27] The increase of choline kinase in Hepa 1c1c7 cells is produced by an ARE-dependent inducer, β-naphthoflavone, but not by an XRE-dependent inducer, benzo[*a*]pyrene or tetrachlorodibenzo-*p*-dioxin,[46] which suggests a role of the ARE. In addition, the ARE is also responsive to hydrogen peroxide.[44] Bile acids can induce an increase in reactive oxygen species.[42]

Of course, it is not known whether transcription from this site is similar or different in drug-treated liver and colon mucosa. This problem must be resolved to understand the function of choline kinase in cancer growth and the mechanism of the increased choline kinase activity. The increase in the choline kinase α mRNA cannot be explained by the elevation of transcription alone. Other mechanisms such as transcription stoppage[21] may also function. The roles of different isozymes, such as choline kinase β, in carcinogenesis of colon mucosa also represent an interesting future problem.

REFERENCES

1) Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smith, A. M. and Bos, J. L. Genetic alterations during colorectal-tumor development. *N. Engl. J. Med.*, **319**, 525–532 (1988).

2) Goldberg, D. M. and Diamandis, E. P. Models of neoplasia and their diagnostic implications: a historical perspective. *Clin. Chem.*, **39**, 2360–2374 (1993).

3) Weber, G. Biochemical strategy of cancer cells and the design of chemotherapy: G. H. A. Clowes memorial lecture. *Cancer Res.*, **43**, 3466–3492 (1983).

4) Schapira, F. Resurgence of fetal isozymes in cancer: study of aldolase, pyruvate kinase, lactic dehydrogenase, and beta-hexosaminidase. *Isozymes Curr. Top. Biol. Med. Res.*, **5**, 27–75 (1981).

5) Denison, M. S., Fisher, J. M. and Whitlock, J. P., Jr. The DNA recognition site for the dioxin-Ah receptor complex. Nucleotide sequence and functional analysis. *J. Biol. Chem.*, **263**, 17221–17224 (1988).

6) Talalay, P., De Long, M. J. and Prochaska, H. J. Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. *Proc. Natl. Acad. Sci. USA*, **85**, 8261–8265 (1988).

7) Prochaska, H. J., De Long, M. J. and Talalay, P. On the mechanisms of induction of cancer-protective enzymes: a unifying proposal. *Proc. Natl. Acad. Sci. USA*, **82**, 8232–8236 (1985).

8) Hernández-Alcoceba, R., Saniger, L., Campos, J., Núñez, M. C., Khaless, F., Gallo, M. A., Espinosa, A. and Lacal, J. C. Choline kinase inhibitors as a novel approach for antiproliferative drug design. *Oncogene*, **15**, 2289–2301 (1997).

9) Druckrey, H., Preussmann, R., Matzkies, F. and Ivankovic, S. Selektive Erzeugung von Darmkrebs bei Ratten durch 1,2-Dimethyl-hydrazin. *Naturwissenschaften*, **54**, 285–286 (1967).

10) Fiala, E. S. Investigations into the metabolism and mode of action of the colon carcinogens 1,2-dimethylhydrazine and azoxymethane. *Cancer*, **40**, 2436–2445 (1997).

11) Kent, C. Regulation of phosphatidylcholine biosynthesis. *Prog. Lipid Res.*, **29**, 87–105 (1990).

12) Kennedy, E. P. Biosynthesis of complex lipids. *Fed. Proc.*, **20**, 934–940 (1961).

13) Abdellatif, M., MacLellan, W. R. and Schneider, M. D. p21 Ras as a governor of global gene expression. *J. Biol. Chem.*, **269**, 15423–15426 (1994).

14) Wiesmüller, L. and Wittinghofer, F. Signal transduction pathways involving *ras*. Mini review. *Cell. Signal.*, **6**, 247–267 (1994).

15) Barbacid, M. *ras* oncogenes: their role in neoplasia. *Eur. J. Clin. Invest.*, **20**, 225–235 (1990).

16) Birchmeier, C., Broek, D. and Wigler, M. *ras* proteins can induce meiosis in *Xenopus* oocytes. *Cell*, **43**, 615–621 (1990).

17) Lacal, J. C. Diacylglycerol production in *Xenopus laevis* oocytes after microinjection of p21*ras* proteins is a consequence of activation of phosphatidylcholine metabolism. *Mol. Cell Biol.*, **10**, 333–340 (1990).

18) Ratnam, S. and Kent, C. Early increase in choline kinase activity upon induction of the H-*ras* oncogene in mouse fibroblast cell lines. *Arch. Biochem. Biophys.*, **323**, 313–322 (1995).

19) Teegarden, D., Taparowsky, E. J. and Kent, C. Altered phosphatidylcholine metabolism in C3H10T1/2 cells transfected with the Harvey-*ras* oncogene. *J. Biol. Chem.*, **265**, 6042–6047 (1990).

20) Lacal, J. C., Moscat, J. and Aaronson, S. A. Novel source of 1,2-diacylglycerol elevated in cells transformed by Ha-*ras* oncogene. *Nature*, **330**, 269–272 (1987).

21) Uchida, T. Stimulation of phospholipid synthesis in HeLa cells by epidermal growth factor and insulin, activation of choline kinase and glycerophosphate acyltransferase. *Biochim. Biophys. Acta*, **1304**, 89–104 (1996).

22) Cuadrado, A., Carnero, A., Dolfi, F., Jiménez, B. and Lacal,

J. C. Phosphorylcholine, a novel second messenger essential for mitogenic activity of growth factors. *Oncogene*, **8**, 2959–2968 (1993).

23) Macara, I. G. Elevated phosphocholine concentration in *ras*-transformed NIH 3T3 cells arises from increased choline kinase activity, not from phosphatidylcholine breakdown. *Mol. Cell. Biol.*, **9**, 325–328 (1989).

24) Warden, C. H. and Friedkin, M. Regulation of choline kinase activity and phosphatidylcholine biosynthesis by mitogenic growth factors in 3T3 fibroblasts. *J. Biol. Chem.*, **260**, 6006–6011 (1985).

25) Nakagami, K., Uchida, T., Ohwada, S., Koibuchi, Y., Suda, Y., Sekine, T. and Morishita, Y. Increased choline kinase activity and elevated phosphocholine levels in human colon cancer. *Jpn. J. Cancer Res.*, **90**, 419–424 (1999).

26) Uchida, T. Immunologically and enzymatically distinct rat choline kinase isozymes. *J. Biochem.* (*Tokyo*), **116**, 1241–1250 (1994).

27) Uchida, T. Regulation of choline kinase R, analyses of alternatively spliced choline kinases and the promoter region. *J. Biochem.* (*Tokyo*), **116**, 508–518 (1994).

28) Traynor, O. J., Costa, N. L. and Wood, C. B. A scanning electron microscopy study of changes in the colonic mucus layer during chemical carcinogenesis. *Cancer*, **51**, 1847–1853 (1983).

29) Koibuchi, Y., Iino, Y., Uchida, T., Nagasawa, M. and Morishita, Y. Effects of estrogen and tamoxifen on the MAP kinase cascade in experimental rat breast cancer. *Int. J. Oncol.*, **11**, 583–589 (1997).

30) Uchida, T. and Yamashita, S. Purification and properties of choline kinase from rat brain. *Biochim. Biophys. Acta*, **1043**, 281–288 (1990).

31) Uchida, T. A novel high-molecular mass mammalian ethanolamine kinase. *Biochim. Biophys. Acta*, **1349**, 13–24 (1997).

32) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).

33) Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159 (1987).

34) Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. and Rutter, W. J. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*, **18**, 5294–5299 (1979).

35) Uchida, T. and Yamashita, S. Molecular cloning, characterization, and expression in *Escherichia coli* of a cDNA encoding mammalian choline kinase. *J. Biol. Chem.*, **267**, 10156–10162 (1992).

36) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254 (1976).

37) UKCCR guidlines for the welfare of animals in experimental neoplasia. *Br. J. Cancer*, **58**, 109–113 (1988).

38) Kuge, O., Nishijima, M. and Akamatsu, Y. Phosphatidylserine biosynthesis in cultured Chinese hamster ovary cells. III. Genetic evidence for utilization of phosphatidylcholine and phosphatidylethanolamine as precurors. *J. Biol. Chem.*, **261**, 5795–5798 (1986).

39) Voelker, D. R. and Kennedy, E. P. Cellular and enzymatic synthesis of sphingomyelin. *Biochemistry*, **21**, 2753–2759 (1982).

40) Exton, J. H. Phosphatidylcholine breakdown and signal transduction. *Biochim. Biophys. Acta*, **1212**, 26–42 (1994).

41) Geilen, C. C., Wieder, T., Boremski, S., Wieprecht, M. and Orfanos, C. E. c-Ha-*ras* oncogene expression increases choline uptake, CTP:phosphocholine cytidylyltransferase activity and phosphatidylcholine biosynthesis in the immortalized human keratinocyte cell line HaCaT. *Biochim. Biophys. Acta*, **1299**, 299–305 (1996).

42) Craven, P. A., Pfanstiel, J. and DeRubertis, F. R. Role of reactive oxygen in bile salt stimulation of colonic epithelial proliferation. *J. Clin. Invest.*, **77**, 850–859 (1986).

43) Fujisawa-Sehara, A., Yamane, M. and Fujii-Kuriyama, Y. A DNA-binding factor specific for xenobiotic responsive elements of P-450c gene exists as a cryptic form in cytoplasm: its possible translocation to nucleus. *Proc. Natl. Acad. Sci. USA*, **85**, 5859–5863 (1988).

44) Rushmore, T. H., Morton, M. R. and Pickett, C. B. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J. Biol. Chem.*, **266**, 11632–11639 (1991).

45) Renton, K. W. and Dickson, G. The prevention of acetaminophen-induced hepatotoxicity by the interferon inducer poly(rI. rC). *Toxicol. Appl. Pharmacol.*, **72**, 40–45 (1984).

46) Paulson, B. K., Porter, T. J. and Kent, C. The effect of polycyclic aromatic hydrocarbons on choline kinase activity in mouse hepatoma cells. *Biochim. Biophys. Acta*, **1004**, 274–277 (1989).