Vol. 163, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1989 Pages 406-411

LEVELS OF PROTEIN KINASE C ACTIVITY IN HUMAN GASTROINTESTINAL CANCERS*

Yu Hashimoto[1,2], Kazuhiro Chida[1], Ming Huang[1], Masafumi Katayama[2], Tetsuro Nishihira[2] and Toshio Kuroki[1,3]

[1]Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108, Japan

[2]Second Department of Surgery, Tohoku University School of Medicine, Seiryo-cho, Sendai 980, Japan

Received July 19, 1989

Summary The protein kinase C (PKC) activities of tumor tissue and adjacent normal mucosa of human cancers of the esophagus (8 cases), stomach (1 case) and colon (3 cases) were measured. Considerable variations were found in the activity of PKC and in its subcellular distribution in these cancers. The PKC activities of the membrane and cytosolic fractions of the eight esophageal cancers were, however, similar to those of the adjacent normal mucosa: the average PKC activities of the tumor tissues and normal mucosa were 7.5 and 8.3 pmol/min/mg protein, respectively, in their membrane fractions and 7.9 and 7.8 pmol/min/mg protein, respectively, in their cytosolic fractions.

Introduction PKC is a phospholipid- and calcium-dependent protein kinase originally isolated by Nishizuka and his colleagues (1). DG, a product of signal-induced inositol phospholipid breakdown, greatly increases the affinity of PKC for phospholipids and calcium at a physiological level, indicating that PKC has a crucial role in signal transduction in the cell membrane mediated by phosphatidylinositol turnover (2).

*Supported in part by a Grant-in-Aid for Special Project Research, Cancer-Bioscience, from the Ministry of Education, Science and Culture of Japan.

[3]To whom all correspondence should be addressed.

Abbreviations: PKC, protein kinase C; DG, diacylglycerol; TPA, 12-O-tetradecanoylphorbol-13-acetate; PS, phosphatidylserine.

0006-291X/89 $1.50

PKC is implicated in tumor promotion, because it acts as a receptor for phorbol ester tumor promoters, e.g. TPA and related compounds, and because PKC is activated by the binding of these tumor promoters. We have been working on the activation, down-regulation and target proteins of tumor promotion *in vivo* and *in vitro*. We found that TPA causes rapid translocation of PKC from the cytosol to the membrane fraction in transformable cells in culture and in mouse skin *in vivo* (3, 4). PKC activated by translocation is subsequently down-regulated by proteolytic cleavage of the PKC molecule (5). Several phosphorylated proteins have been identified in these cells: these include a membrane protein of Mr 80,000 commonly found in cultured cell lines and proteins of Mr 40,000 and 34,000 in mouse epidermis *in vivo* (3, 4).

PKC may also be implicated in phenotypic expression of transformed cells. In cells transformed by the c- or v-*ras* gene, elevated levels of DG and/or inositol phosphate(s) have been found (6-10). Moreover, we have reported that in *ras*-transformed 208F cells of rats, phosphatidylinositol metabolism is stimulated through imbalance of the kinase activities concerned, leading to accumulation of DG, and thereby activation and down-regulation of PKC (10). In view of these considerations, it was of particular interest to examine the levels and localization of PKC activity in normal mucosa and tumors of the gastrointestinal tract of humans. In this study, we found that esophageal tumor tissues contain almost the same PKC activity as the adjacent normal mucosa.

Materials and Methods

Chemicals. Histone (type III-S), ATP, PS and diolein were purchased from Sigma, MO, USA; [γ-^{32}P]ATP (specific activity, 20-40 Ci/mmol) was obtained from New England Nuclear, MA, USA.
Tissues. Surgically resected materials were collected from the University Hospital of Tohoku University, Sendai and Ookuchi Hospital, Yokohama. Intact tumor tissues without ulcerated or necrotic portions were used. The submucosa and muscularis were removed from adjacent normal mucosa. Tissues were cut into small fragments and frozen in dry ice until examined. Portions of the same tissues was processed for routine histological examination.
Assay of PKC activity. Tissue samples were homogenized and sonicated in 10 ml of extraction buffer consisting of 5 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride, 5 mM 2-mercapto-ethanol and 20 nM Tris-HCl, pH 7.5. Tissue homogenates were centrifuged at 100,000 x g for 1 h at 4°C, and the supernatant was used as the cytosolic fraction. The pellet was solubilized in 5 ml of extraction buffer containing 1% Triton X-100 for 1 h

at 4°C, and then centrifuged at 100,000 x g at 4°C for 1h, and the resulting supernatant was used as the membrane fraction. The cytosolic and particulate fractions were further purified by DEAE-cellulose (DE52, Whatman) column (0.5 ml) chromatography, PKC activity being eluted with 0.3 M NaCl.

Protein kinase activity was assayed in reaction mixture consisting of 20 mM Tris-HCl (pH 7.5), 5 mM $MgSO_4$, 200 µg/ml histone, 10 µM [γ-^{32}P]ATP, and 50 µl of enzyme preparation in a final volume of 250 µl. PKC activity was defined as the difference between the ATP-histone phosphotransferase activity in reaction mixture containing 1 mM $CaCl_2$, 64 µM PS, and 1.3 µM diolein (Ca/PS/DG) and in that containing 1 mM EGTA with no Ca/PS/DG. After incubation for 3 min at 30°C, the reaction was stopped by adding 5 ml of 25% trichloroacetic acid, and the radioactivity of the acid-insoluble fraction was counted.

Results and Discussion

Tables 1 and 2 summarize the PKC activities recovered in the membrane and cytosolic fractions of human cancers of the esophagus, stomach and colon and their adjacent normal mucosa. This activity is defined as the ATP-histone phosphotransferase activity stimulated by Ca^{2+}, PS and DG. The tumor tissues in the eight esophageal cancers contained similar PKC activities to those of the adjacent normal mucosa (Table 1). The average PKC activity (in pmol/min/mg protein) of the membrane fraction of these esophageal tumors was 7.5 (SD, 5.3; range, 0.3 -

Table 1. PKC Activity of Normal Mucosa and Tumor Tissue of Esophageal Cancers

Patient (age,sex)	Fraction	PKC Activity[a]	
		Tumor	Normal
1 (81, F)	membrane	15.7	7.6
	cytosol	8.5	6.8
2 (56, M)	membrane	0.3	0.5
	cytosol	0.5	0.6
3 (74, M)	membrane	0.7	4.8
	cytosol	3.2	6.5
4 (60, M)	membrane	7.5	10.8
	cytosol	8.7	6.0
5 (51, M)	membrane	6.6	7.7
	cytosol	11.4	14.0
6 (55, M)	membrane	5.4	11.2
	cytosol	13.4	11.8
7 (76, M)	membrane	14.9	15.8
	cytosol	11.0	9.4
8 (68, M)	membrane	8.9	7.8
	cytosol	6.4	7.6

[a] pmole/min/µg protein, average of three measurements.

Table 2. PKC Activity of Normal Mucosa and Tumor Tissue of Stomach and Colon Cancers

Patient (age,sex)	Fraction	PKC Activity[a]	
		Tumor	Normal
Stomach cancers			
9 (60, M)	membrane	3.0	19.8
	cytosol	31.6	7.6
Colon cancers			
10 (66, F)	membrane	2.8	11.7
	cytosol	85.7	16.7
11 (57, F)	membrane	12.8	19.8
	cytosol	15.0	19.5
12 (79, F)	membrane	8.8	22.2
	cytosol	13.7	11.4

[a] pmole/min/μg protein, average of three measurements.

15.7), whereas that of the adjacent normal mucosa was 8.3 (SD, 4.3; range, 0.5 - 15.8). The PKC activities in the cytosolic fractions were very similar to those in the membrane fractions: 7.9 (SD, 4.1; range, 0.5 - 13.4) for tumor tissues and 7.8 (SD, 3.8; range, 0.6 - 14.0) for normal mucosa.

The PKC activities in stomach and colon cancers were rather higher than those in esophageal cancers. Because few cases were examined, values were not compared in terms of tumor tissue *vs.* normal mucosa or the cytosolic *vs.* the membrane fraction.

Considerable variations were found in the subcellular distributions of PKC activity both in tumor tissues and the adjacent normal mucosa and in different patients. Similar large variations have been observed in the PKC activities of various cell lines (11) and in human cancers of the colon, stomach and breast (12, 13). These variations may be mainly attributable to variations in PKC activity itself or in specific expression of its subtypes. The activity may also be influenced by other factors, such as cell-specific substrate specificity, the presence of inhibitors or activators and the proportion of stroma cells in the tumor tissues.

In a previous study (11), we examined the activities of PKC and the bindings to phorbol-12,13-dibutyrate of 41 cell lines derived from normal and malignant tissues of experimental animals and humans. In general, values were found to be high in normal or untransformed cells and low in malignant or

transformed cells. Wolfman and Macara (8) observed activation of PKC in ras-transformed NIH/3T3 cells, using phosphorylation of an 80,000 protein as a marker. Moreover, we have found that PKC is translocated, activated and down-regulated in ras-transformed 208F rat fibroblasts resulting in lower total activity of PKC, but higher membrane-associated activity in these transformed cells (10). On examination of 22 cell lines derived from human cancers, Hirai et al. (14) found that lung cancer cells often exihibited significantly higher PKC activity than other types of cancer cells.

There are three previous reports on PKC activity in human cancer tissues. Guillem et al. (12) reported that the PKC activities of the cytosolic and particulate fractions of human colon cancers were both reduced compared with those of adjacent normal mucosas. In contrast, Lim et al. (13) found that the PKC activities of the cytosolic and particulate fractions of human stomach and breast tumors were higher than those of adjacent normal tissues. Recently, O'Brian et al. (15) reported elevated expression of total PKC activity in breast tumor biopsies relative to normal tissues obtained from the same patients. In the present study, however, we found no difference in the PKC activities or distributions of PKC in esophageal cancers and adjacent normal mucosa. Further data are needed to determine whether the levels of PKC activity and malignancies of human tumors are related.

References

1. Inoue, M., Kishimoto, A., Takai, Y., and Nishizuka, Y. (1977) J. Biol. Chem. 252, 7610-7616.
2. Nishizuka, Y. (1984) Nature 308, 693-698.
3. Chida, K., Hashiba, H., Sasaki, K., and Kuroki, T. (1986) Cancer Res. 46, 1055-1062.
4. Chida, K., Yamada, S., Kato, N., and Kuroki, T. (1988) Cancer Res. 48, 4018-4023.
5. Chida, K., Kato, N., and Kuroki, T. (1986) J. Biol. Chem., 261, 13013-13018.
6. Fleischman, L. F., Chahwala, S. B., and Cantley, L. (1986) Science 231, 407-410.
7. Preiss, J., Loomis, C. R., Bishop, W. R., Stein, R., Niedel, J. E., and Bell, R. M. (1986) J. Biol. Chem., 261, 8597-8600.
8. Wolfman, A., and Macara, I. G. (1987) Nature 325, 359-361.
9. Lacal, J. C., Moscat, J., and Aaronson, S. A. (1987) Nature 330, 269-272.
10. Huang, M., Chida, K., Kamata, N., Nose, K., Kato, M., Homma, Y., Takenawa, T., and Kuroki, T. (1988) J. Biol. Chem., 263, 17975-17980.

11. Chida, K., Kato, N., Yamada, S., and Kuroki, T. (1988) Biochem. Biophys. Res. Commn. 157, 1-8.
12. Guillem, J.G., O'Brian, C.A., Fitzer, C. J., Forde, K. A., LoGerfo, P., Treat, M., and Weinstein, I. B. (1987) Cancer Res. 47, 2036-2039.
13. Lim, I. K., Lee, D. J., Lee, K. H., and Yun, T. K. (1987) Yonsei Med. J. 28, 255-260.
14. Hirai, M., Gamou, S., Kobayashi, M., and Shimizu, N. (1989) Jpn. J. Cancer Res. 80, 204-208.
15. O'Brian, C. A., Vogel, V. G., Singletary, S. E., and Ward, N. E. (1989) Cancer Res. 49, 3215-3217.