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The enhanced ^{32}P labeling of CDP-diacylglycerol in c-*myc* gene expressed human kidney cancer cells

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Correlation between c-*myc* gene expression and phosphatidylinositol (PI) metabolism was studied using seven human kidney cancer cell lines. We found that the exceptional incorporation of ^{32}P into CDP-diacylglycerol (CDP-DG) was observed in only two cell lines, in which c-*myc* expression was increased among seven cell lines tested. In these two cell lines, PI was also labeled. The other five cell lines indicated the accumulation of radioactive PI, whereas CDP- DG was unlabeled.

c-*myc* gene; Phosphatidylinositol metabolism; CDP-diacylglycerol; (Kidney cancer cell)

1. INTRODUCTION

Recent studies have suggested that several steps in the PI cycle may be involved in the action of mitogenic signaling [1]. Activation of cellular oncogenes is known to result in cell transformation. Here, we attempted to determine whether c-*myc* expression in cell proliferation is related to the PI cycle. We have measured levels of c-*myc* RNA, as well as that of PI turnover metabolites, with 7 cell lines established from human kidney cancers.

Among the 7 cell lines, only two were found to have increased c-*myc* expression. Furthermore, only these two cell lines showed a very marked enhancement of ^{32}P labeling in CDP-DG, when these cells were incubated with $^{32}P_i$. The results support the idea that the PI cycle might be associated with the activation of c-*myc* gene expression.

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Abbreviations: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PG, phosphatidylglycerol; TLC, thin-layer chromatography; HPTLC, high-performance TLC

2. MATERIALS AND METHODS

2.1. *Materials*

Carrier-free [^{32}P]orthophosphate ($^{32}P_i$, 1 mCi/ml) and [^{32}P]dCTP (3000 Ci/mmol) were purchased from Amersham. Seven human renal cancer cell lines were used in this study. YCR-1 and RC-YAMATO cells were established in our laboratory; KN-41, 5873T and C-6-3 cells were supplied by Kanazawa University (Kanazawa, Japan), Duke University (NC, USA) and Osaka Adult Disease Center (Osaka), respectively. ACHN and CAKI-2 were obtained from the American Type Culture Collection (ATCC). All of these cell lines were established from the tumors of human kidney cancer. Human promyelocytic leukemia cell line, HL-60 was obtained from the Japanese Cancer Research Bank. They were subcultured and maintained with Ham's F-12 medium (Gibco) supplemented with 10% fetal calf serum (Hiclone

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Laboratory, USA). The plasmid containing the 2nd and 3rd exons of human c-*myc* was obtained from ATCC.

2.2. *Labeling of cells with $^{32}P_i$ and detection of CDP-DG*

Labeling of cells with $^{32}P_i$ was carried out according to Sugimoto et al. [2]. Subsequent extraction and analysis of ^{32}P-labeled phospholipids were carried out as in [3]. The identity of radiolabeled phospholipids was confirmed by co-chromatography with authentic samples.

2.3. *RNA extraction and Northern blotting*

Cytoplasmic RNA was extracted from the cultured kidney cancer cell lines using NP-40 as described by Lee et al. [4]. 20 μg RNA from each cell line was isolated and submitted to the RNA gel study. Northern blot experiments were performed in the usual manner.

3. RESULTS

All cell lines showed typical growth characteristics of cancer cells, i.e. anchorage-independent growth and loss of contact inhibition. The population doubling time of these cells was found to be 17.0, 18.0, 20.4, 21.6, 22.0, 31.2, 32.4 and 38.0 h in KN-41, YCR-1, RC-YAMATO, ACHN, 5873T, CAKI-2 and C-6-3 cells, respectively. Obviously, the first half of the group show a shorter population doubling time (about 20 h), whereas that of the others is 1.5-times longer (more than 30 h).

As shown in fig.1, c-*myc* expression was increased in the two cell lines, YCR-1 and KN41, which showed an unusually large amount of accumulation of radioactivity in the band of CDP-DG. The other cell lines, ACHN, RC-YAMATO, CAKI-2, C-6-3 and 5873T, which did not show increased c-*myc* expression, indicated no accumulation of the radioactivity in the position of CDP-DG.

Two-dimensional TLC analysis was also applied to ^{32}P-labeled phospholipids and it was found that ^{32}P incorporation into CDP-DG and PG was increased markedly in KN41 and YCR-1, after 60 min incubation with $^{32}P_i$, while both CDP-DG and PG were unlabeled or weakly labeled in other cell lines (fig.2a,b).

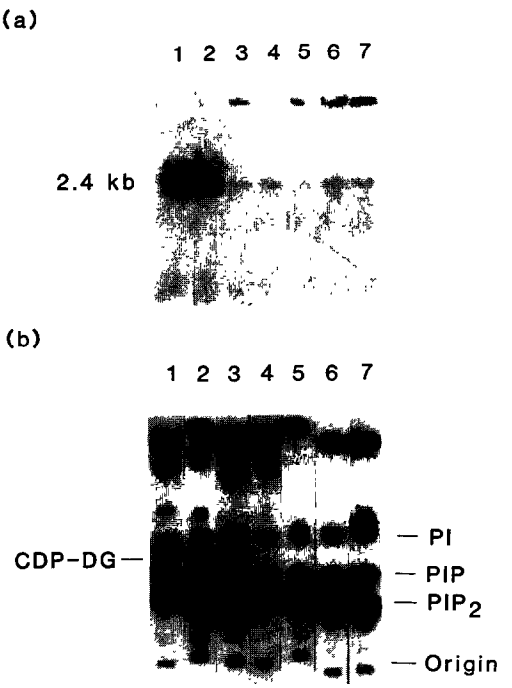

Fig.1. Comparison between c-*myc* gene expression and the ^{32}P-labeling of phospholipids for 7 kinds of cell lines. (a) Autoradiograph of Northern blot with cytoplasmic RNA. (b) Autoradiograph of one-dimensional HPTLC pattern of ^{32}P-labeled phospholipids. Lanes: 1, KN-41; 2, YCR-1; 3, ACHN; 4, 5873T; 5, C-6-3; 6, CAKI-2; 7, RC-YAMATO.

The rate of ^{32}P incorporation into CDP-DG was measured at various incubation times, and it was found that the rate of radiolabeled CDP-DG accumulation in the cell lines, YCR-1 and KN41, was greater than that of ^{32}P-labeled PI accumulation in other cell lines (fig.2c,d), although the rates of accumulation of labelled PIP and PIP_2 are almost the same for all cell lines tested. These results suggest that the PI cycle is activated in c-*myc* expressed cell lines.

We examined c-*myc* expression and ^{32}P labeling of phospholipids in HL-60 leukemia cells for comparison. A significant increase in the level of c-*myc* RNA was evident, but labelled CDP-DG was not observed in the cell line (not shown). Other oncogenes, c-*fos*, c-*fms* and c-Ha-*ras*, were found to be expressed equally but weakly among the cell lines tested.

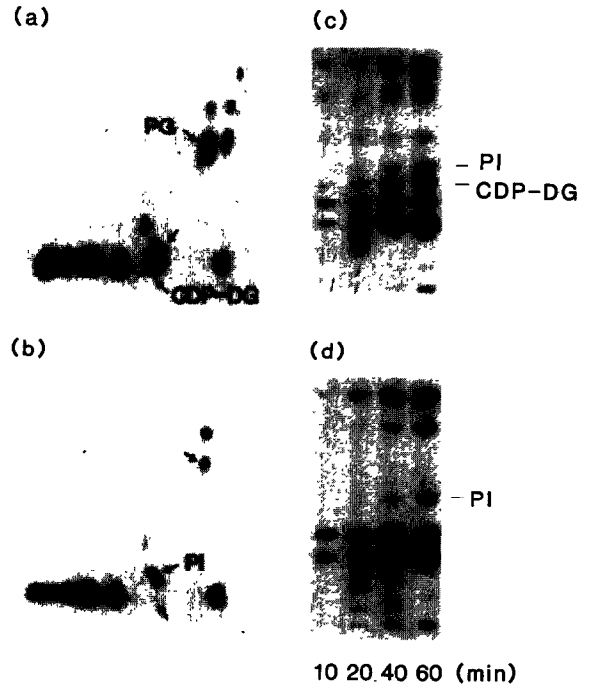

Fig.2. Autoradiograph of two-dimensional TLC pattern of ^{32}P-labeled phospholipids for (a) KN-41 and (b) 5873T cells. Autoradiograph of one-dimensional HPTLC patterns of phosphorylated phospholipids for various incubation times (10, 20, 40 and 60 min) for KN-41 (c) and for 5873T (d).

4. DISCUSSION

In this paper, abnormal enhancement of ^{32}P incorporation into CDP-DG has been shown in the c-*myc* expressed human kidney cancer cell lines, KN-41 and YCR-1. We have also examined a typical c-*myc* expressed human promyelocytic leukemia cell line, HL-60, and have not observed increased radiolabeling of CDP-DG. The discrepancy in CDP-DG accumulation between KN-41 (YCR-1) and HL-60 can be ascribed to the difference in genetic characteristics among those cell lines tested. The high level of c-*myc* mRNA in HL-60 cells, originating from hematopoietic cells, is caused by c-*myc* gene amplification [5,6]. On the other hand, none of the renal cancer cell lines in this study showed c-*myc* gene amplification (not shown). Therefore, we prefer to emphasize that accumulation of radioactive CDP-DG in c-*myc* expressed cells should be compared among cell lines with one kind of tumor, having a very similar genetic (and probably metabolic) origin.

The abnormally increased labeling of CDP-DG in kidney cancer cells could be explained as being due to the limitation of CDP-DG inositol phosphatidyltransferase by the lack of inositol substrate as was proposed by Frinkel et al. [7] with rat pancreatic islets. Frinkel et al. observed that a chronic low level of inositol, brought about by glucose stimulation, enhanced the labeling of CDP-DG accompanied by that of PG. Hauser and Eichberg [8] noted that propranolol caused a stimulation in the ^{32}P labeling of PG and CDP-DG of isolated rat pineal organ in the absence of inositol [8]. Similar results are demonstrated in c-*myc* activated kidney cancer cell line as shown in fig.2a. The accumulation of DG was also reported in GH3 cells under conditions of chronic low inositol [9]. This could explain the activation of the c-*myc* gene, since mitogenes that activate c-*myc* expression also activate PI turnover and increased DG concentration [10,11].

Although there are conflicting data [12,13], intracellular Ca^{2+} and/or C-kinase might be another candidate for the regulation of c-*myc* activation as suggested by Kaibuchi et al. [14]. Further experiments are necessary to confirm the possibility that abnormal PI turnover, as shown in this report, might be involved in the activation of the c-*myc* gene. As discussed above, the mechanism which activates c-*myc* transcription is, despite many studies, largely unknown, but we suggest that it does point to a relationship between c-*myc* activation and the inositide pathway.

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