ENHANCEMENT OF PHOSPHOLIPID METHYLATION IN CULTURED HAMSTER CELLS BY VIRAL TRANSFORMATION

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1. Introduction

Cell transformation by oncogenic viruses results in loss of growth control accompanied by modification in growth rate, substrate adherence [1,2], and cellular membrane microviscosity [3,4]. Different authors pointed out the role of phospholipid polar head groups in cell-substrate adherence [5], intercellular contact [6] or in cellular membrane fluidity [7].

Phospholipid methylation in proliferation of lymphocytes induced by lectins [8] was enhanced prior to DNA synthesis, and appeared to be an essential step in cell proliferation, since inhibitors of methylation prevented lymphocytes transformation. Thus, phospholipid methylation seemed to be an important event in the regulation of cell growth, at least in the case of lymphocytes.

Here, phospholipid methylation was measured in normal and PY or SV40-transformed hamster fibroblasts. Experiments were carried out in situ with L-[^{14}C]methionine or in vitro with *S*-[^{14}C]-adenosyl methionine. It was demonstrated that phospholipid methylation was enhanced by viral transformation.

2. Materials and methods

2.1. *Cell culture*

The cell lines studied were EHB cells, established hamster fibroblasts, and EHSVi cells, SV40-transformed hamster fibroblasts [9], BHK 21/C 12 and virus-transformed PY-BHK 21 from Flow Labs. Seeded at 3×10^4 cells/cm^2, cells were cultured in 25 cm^2 Corning flasks, with 5 ml minimum essential medium containing 10% of foetal calf serum (Gibco). All experiments were performed in exponential growth phase, 2 days after seeding.

2.2. *Incorporation of L-[^{14}C]methionine into phospholipids*

Incorporation of 2 μCi/ml L-[*methyl*-^{14}C]methionine (55 mCi/mmol, CEA, France) was followed as a function of time. After incubation at 37°C, cells were washed 3 times with a phosphate-buffered solution, harvested with rubber-policemen, centrifuged and resuspended in 9 g NaCl/l. Protein determination was done by the Lowry method and phospholipid separation was performed by chromatography on silica gel plates with chloroform/methanol/H_2O (65:25:4, by vol.) after direct application of an aliquot of the cell suspension as in [10]. After autoradiography, the labelled phospholipids lysophosphatidylcholine and phosphatidylcholine, identified with known standards, were cut out and the radioactivity counted by liquid scintillation in an Intertechnique. Results are expressed in mol methyl groups incorporated/mol phospholipids.

2.3. *Phospholipid methylation*

For the obtention of the enzyme extract, cells in exponential growth phase were harvested with rubber-policemen, centrifuged, resuspended in distilled water, frozen and thawed 3 times.

The method in [11] was applied for the determination of phospholipid methylation. The incubation mixture (final vol. 50 μl) contained phosphate buffer 0.05 M (pH 8), $MgCl_2$ 5×10^{-3} M when added, 2×10^5 cpm *S*-[^{14}C-*methyl*]adenosyl-methionine, NEN, 57.6 mCi/mmol diluted with non-labelled substrate from Sigma (final conc. substrate 2×10^{-4} M), and ~80 μg cellular proteins. After incubation for 1 h,

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the reaction products were separated by chromatography on silica gel plates with chloroform/methanol/H_2O/acetic acid (75:45:6:12, by vol.), identified with standards from Gibco, revealed by autoradiography, and counted by liquid scintillation.

3. Results and discussion

Data obtained by in situ incorporation of L-[^{14}C]-methionine are given in fig.1. Both transformed cell lines EHSVi and PY-BHK exhibited increased phospholipid methylation as compared to their normal counterparts. The two major labelled phospholipids obtained in these experiments were identified to be lysophosphatidylcholine and phosphatidylcholine; sphingomyelin, the third choline-containing phospholipid, accounted for ~3% of the total radioactivity. The ratio lysophosphatidylcholine/phosphatidylcholine was increased ~3-fold in the 2 transformed cell lines: in these cells, 70–90% of the radioactivity were recovered in lysophosphatidylcholine.

It thus appeared that viral transformation was accompanied by increased phospholipid methylation.

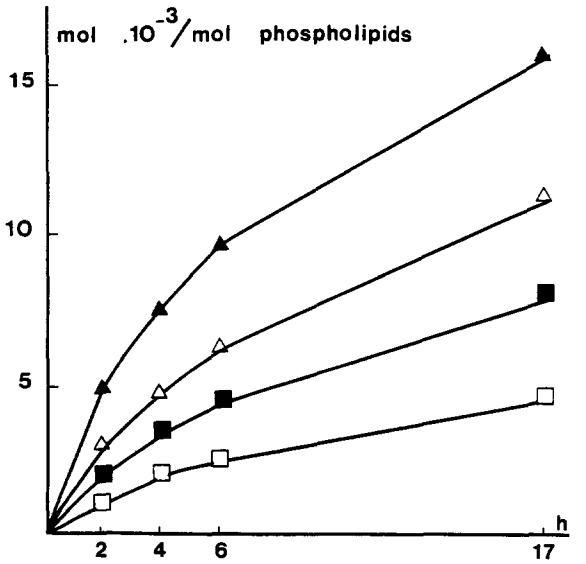

Fig.1. Incorporation of L-[*methyl*-^{14}C]methionine into phospholipids of normal and virus-transformed hamster fibroblasts: EHB (□)/EHSVi (△) and BHK (■)/PY-BHK (▲). Abscissa: incubation time (h). Ordinate: mol 10^{-3} methyl groups incorporated/mol phospholipids. Cells were in exponential growth phase and labelling was performed with 2 μCi/ml of L-[*methyl*-^{14}C]methionine 55 mCi/mmol.

Furthermore, the phosphatidylcholine molecular species arising from phosphatidylethanolamine were more rapidly degraded to lysophosphatidylcholine in transformed cells. Since phosphatidylcholine synthesized by methylation was enriched in polyunsaturated fatty acids such as arachidonic acid the precursor of prostaglandins [12], our results are consistent with the increased prostaglandins synthesis induced by viral transformation in [13,14]. In lymphocytes, stimulation of division by mitogenic lectins induced a transient increase in methylated phospholipids [8], the phosphatidylcholine molecules thus formed gave rise to arachidonic acid and lysophosphatidylcholine by activation of phospholipase A_2.

Determination of phospholipid transmethylases activities was carried out on cell extracts and the results are given in table 1. In our experimental conditions, the distribution of radioactivity among the 3 labelled phospholipids was: phosphatidylmonomethylethanolamine 30%; phosphatidyldimethylethanolamine 40%; and phosphatidylcholine 30%. Mg^{2+} clearly enhanced the enzymatic activities. Moreover, the transformed cell lines exhibited increased transmethylase activities as compared to their normal counterparts, in the presence or absence of Mg^{2+}. Thus, the results obtained with in situ incorporation of L-[^{14}C]-methionine have been confirmed. Comparison of the 2 normal cells EHB and BHK shows that the BHK cells presented higher activity than EHB cells: this difference might be due to the different origin of the 2 cell lines.

Phospholipid methylation has been related to calcium transport: increased Ca^{2+} efflux from erythro-

Table 1
Phospholipid methylation measured on cell extracts of normal and transformed hamster fibroblasts

Cell lines	Moles 10^{-12} of methyl groups incorporated into phospholipids/h/mg proteins	
	without Mg^{2+}	with Mg^{2+}
EHB	150 ± 30	230 ± 40
EHSVi	780 ± 80	1490 ± 210
BHK	310 ± 30	420 ± 50
BHK-PY	840 ± 90	1600 ± 150

The incubation mixture contained phosphate buffer 0.05 M (pH 8), $MgCl_2$ 5 × 10^{-3} M when added, 2 × 10^5 dpm of *S*-[^{14}C-*methyl*]adenosyl-methionine (final conc. 2 × 10^{-4} M) and ~80 μg cellular proteins. Mean of 4 expt in duplicate ± SD

cytes has been associated with methylation of phospholipids [15]; on the other hand, according to [16], calmodulin enhanced phospholipid methylation. In this regard, our results might be related to the increase in Ca^{2+} transport induced by viral transformation [17]. Other cellular membrane properties, such as microviscosity [8], β-adrenergic receptor–adenylate cyclase coupling [7] or chemotaxis [18,19] have been shown to be dependant of phospholipid methylation. Thus, modifications in phospholipid methylation might be involved in several perturbations of cell membrane structure and function observed after oncogenic transformation.

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