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THE EFFECT OF db-cAMP ON THE GENE EXPRESSION OF CALMODULIN AND CYTOSKELETON IN THE TRANSFORMED CELLS

Liu Huitu 柳惠图 Wang Duanshun 王端顺 Zhang Hongqing 张鸿卿
Xue Shao bai 薛绍白 You Jinsong 游劲松 Du Chunying 杜春英 Wang Xiaoliang 王晓良

Department of Biology, Beijing Normal University

We have demonstrated that the distribution of microtubules (MT), microfilaments (MF) and fibronectin (FN) were diminished, while the gene expression of the calmodulin and c-*fos* enhanced in the transformed $C_3H_{10}T_{1/2}$ cells. After treatment with 1 mM db-cAMP for 1 hour and 2 hours, there was an early and repidly reduced in gene expression of Calmodulin and c-*fos* respectively. After db-cAMP treatment for 4 — 5 days, the number of capping cells of ConA binding decreased significantly and the cell surface microvilli decreased as well. The growth of treated cells was inhibited markedly. By using $4F_1$ cDNA probe, which is preferentially expressed in G_1 phase, we have found that the db- cAMP treated cells were accumulated at G_1 phase. Of particular interest is the fact that the distribution of microtubules, microfilaments and fibronectin were recovered after treatment with 1 mM db-cAMP for 6 days. It is suggested that the inhibition of proliferation, alteration of phenotype and reco- very of cytoskeleton is transformed cells after treatment with db-cAMP are related to the inhibition of gene expression of Calmodulin.

A voluminous literature has documented the role of cAMP in inhibition of cell proliferation and promotion of cell differentiation.[1,2] The level of cellular cAMP was decreased after transformation.[3] The distribution of cytoskeleton, especially microtubules(MT) and microfilaments (MF) were diminished in transformed cells.[4,5] The cells was rounded. However, db- cAMP promote the recovery of cytoskeleton which is associated with increased adhesion of the cell to the substrate and a more flattened morphology.[6,7] Lockwood suggested that a cAMP- dependent protein kinase system phosphorylate cellular proteins, these phospho-proteins mediate the acquisition of normal morphology and the organization of the cytoskeleton induced in transformed cells by cAMP.[8]

Recently, Calmodulin (CaM) has been demonstrated to be involved in the regulation of the assembly of cytoskeleton and cell proliferation. A cell cycle- dependent increase in Calmodulin content is required for progression of cells from the G_1 to the S phase of the cell cycle, anticalmodulin durgs arrest the cells at the G_1/S boundary.[9,10] It has been demonstrated that the level of Calmodulin is higher in transformed cells than that in normal cells.[11,12] Calmodulin play an important role in regulating the polymerization of the microflaments and microtubules by flipflop swith or CaM-dependent kinase.[11,13,14]

The important role of Cam in cell proliferation and transformation was reported, but until now little consideration has actually been given to the role for CaM in differentiation of transformed cells. In this paper, we examine the correlation between the gene expression of CaM and the assembly of cytoskeleton in transformed $C_3H_{10}T_{1/2}$ cells by db-cAMP and study the effects of CaM on induced differentiation of transformed cells by db-cAMP.

MATERIALS AND METHODS

Cell Culture

Normal and transformed $C_3H_{10}T_{1/2}$ cells were cultured in DMEM supplemented with 15% calf serum. After seeding transformed cells for 24 hours 1 mmol/L db-cAMP and 1 mmol/L theophylline were added in medium. Cells were counted dayly.

Scanning Electron Microscopy

Cells on coverslips were fixed in 2.5% glutaraldehyde and 1% osmic acid respectively, dehydrated in gradient concentrations of ethanol and fried by the critical point method, the coverslips were coated with platinum and observed with scanning electron microscope.

Immunofluorescent Labeling for ConA-receptor

After culture for 4 days, cells on coverslip were incubated with FITC-ConA at 37℃ for 10 minutes and observed with fluorescent microscope.

Indirect Immunofluorescent Staining of Microtubules and Fibronectin

After culture for 6 days, the microtubules immunofluorescent staining were carried out as described previously.[15] The staining procedure for fibronectin as following: cells on coverslip fixed at room temperature for 30 minutes in 3.7% formaldehyde/PBS, incubated with anti-fibronectin antibody and FITC-IgG for 45 minutes at 37℃ respectively, observed with immunofluorescent microscope.

Fluorescent Staining of Microfulaments

After culture for 6 days, cells on coverslip fixed for 10 minutes at room temperature in 3.7% formaldehyde/PBS, placed for 5 minutes in acetone at -20℃, stained by Rhodamine-phalloidine/PBS for 20 minutes at room temperature and observed with immunofluorescent microscope.

cDNA Probe

c-*fos* cDNA purchased from Oncor Inc.. $4F_1$ cDNA was a gift from Prof. R. Baserga of Temple University Medical School USA Calmodulin cDNA was a gift from Prof. Lizhi Hao of Baylor University School of Medicine, USA

RNA Extraction, Northern Blotting and Dot Blot

RNA extraction, Northern blotting and dot blot were carried out as described previously.[16] After expouse of X-film the relative intensity of RNA dot blot was determined quantitatively.

RESULTS

Effect of db-cAMP on the Cell Growth

In comparison with normal cells, the transformed cells grew fast. After seeding for 5 days, number of cells was more than 3 times that of the normal cells. Growth of transformed cells treated with db-cAMP (1 mM) for 5 days was inhibited significantly. The number of cells was less