Chinese Journal of Cancer Research 9(1):32-35,1997.

EXPRESSION OF PLACENTAL ALKALINE PHOSPHATASE IN ESOPHAGEAL CANCER CELL LINE Eca109

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The expression and properties of alkaline phosphatase (ALP) in Eca109 cells, a cell line derived from human esophageal cancer were studied with specific inhibition assay and polyacrylamide gel electrophoresis. The results showed that ALP of Eca109 cells was heat stable and was strongly inhibited by L-phenylalanine, but slightly inhibited by urea. Prednisolone could cause dramatic increase in activity of ALP, but no change in ALP isozyme and concomitant increase in lactic dehydrogenase activity were found after prednisolone treatment. The results suggested that placental alkaline phosphatase as an oncodevelopmental gene product could be expressed ectopically by Eca109 cells and prednisolone could specifically induce increase in its activity.

Key words: Esophageal cancer cell line Eca109, Alkaline phosphatase (ALP).

Placental alkaline phosphatase (PLAP) has been shown to be a oncodevelopmental gene product, which is most frequently produced by some human malignant tumors and tumor-derived cell lines, such as ovarian cancer, cervical cancer and testicular cancer.[1,2] It has heretofore not been reported that esophageal cancer or cell line express the enzyme. For this reason, we studied its expression and prednisolone induction in esophageal cancer cell line, Eca109.

MATERIALS AND METHODS

Accepted January 15, 1997

Cell Culture and Prednisolone Treatment

Eca109 cells were obtained from Department of Cell Biology, Cancer Institute, Chinese Academy of Medical Scieces. Cells were cultured at 37 ℃ in Medium 199 supplemented with 20% heated newborn calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were transferred every a week using a mixture of 0.25% trypsin and 0.02% EDTA, and inoculated at a density of 3×10^5/25 cm^2 flask. 24 h after cells transfer, the cells were used for experimentation. Prednisolone treatment was done by adding prednisolone to the medium at a final concentration of 1 μg/ml. Cells grown in regular medium served as controls. After a further growth, the cells were harvested respectively at 12, 24, 48, 72 and 96 h. The harvested cells were washed three times with cold physiological saline by resuspension and centrifugation, then lysed with 0.5% sodium deoxycholate for enzyme assay.

Alkaline Phosphatase (ALP) Activity Assay

Total ALP activity was measured by using disodium phenylphosphate.[3] The specific acitivity was expressed in units per mg protein.

Inhibition Studies

Heat inactivation of ALP was performed by heating samples in a water bath at 65 ℃ for 10 min. L-phenylalanine or urea inhinition was done by adding L-phenylalanine (10 mmol/L) or urea (2 mol/L) to the reaction solution. The remaining activities of ALP

were measured and percentage of inhibition was calculated from control activity.

Gel Electrophoresis

Disc gel electrophoresis was carried out in 10% polyacrylamide with Triton X-100 according to the method of Fishman.[4] Enzyme activity was visualized by staining the gel accoridng to the method of lee[5] with disodium α-naphthylphosphate as substrate and diazo fast blue RR as dey.

Lactic Dehydrogenase (LDH) Activity Assay

LDH was measured according to the published procedure.[3] The specific activity was expressed in units per mg protein.

RESULTS

Properties of ALP in Eca109 Cells

The ALP content, degree of L-phenylalanine or urea inhibition and electrophoretic separation of isozyme was observed in regular cultured Eca109 cells. As shown in Table 1, heat treatment of ALP at 65 ℃ for 10 min failed to cause significant loss of activity. The enzyme activity reduced markedly in the present of L-phenylalanine (10 mmol/L) and reduced slightly in the present of urea (2 mol/L). It was inhibited about 80% by L-phenylalanine and 25% by urea. Electrophoresis revealed one sharp band, and the band remained when the heated sample was subjected to electrophoresis (Figure 1). The specific activity of ALP rose from 0.089 to 0.125 during the growth stage (12 to 96 h after experiment) (Table 2).

Effect of Prednisolone on ALP of Eca109 Cells

By contrast to the control cells at indicated time intervals, it was demonstrated that prednisolone cause a progressive increase in ALP specific activity. ALP specific activity showed a tendency to increase at 12 h. The increase became apparent at 24 h ($P<0.01$) and reached a maximum at 72 h. It was 3-fold that of control cells between 48 and 96 h (Table 2). However, there was no significant difference in ALP heat stability and degree of L-phenylalanine or urea inhibition between control and prednisolone treated cells ($P>0.05$) (Table 1). Electrophoresis still showed one sharp band and the band remained after heat treatment (Figure 2).

Table 1. Effect of heat, L-phenylalanine and urea on ALP activity in Eca109 cells

Treatment	Inhibition (%, $\bar{x}\pm s$)		P
	Control cells	Prednisolone treated cells	
Heat (65 ℃, 10 min)	0	0	
L-phenylalanine (10 mmol/L)	80.83±1.57	82.18±2.17	>0.05
Urea (2 mol/L)	25.23±1.12	25.42±1.32	>0.05

Table 2. Effect of prednisolone on the activity of ALP and LDH in Eca109 cells ($\bar{x}\pm s$)

Time (h)	ALP specific activity		P	LDH specific activity		P
	Control cells	Prednisolone treated cells		Control cells	Prednisolone treated cells	
12	0.089±0.002	0.113±0.002	>0.05	12.988±2.766	13.125±2.815	>0.05
24	0.104±0.002	0.255±0.003	<0.01	14.754±2.821	15.047±2.479	>0.05
48	0.112±0.001	0.353±0.002	<0.01	17.175±3.462	17.219±2.987	>0.05
72	0.118±0.001	0.381±0.002	<0.01	20.491±2.931	19.963±3.411	>0.05
96	0.125±0.003	0.380±0.002	<0.01	21.655±3.653	20.982±3.463	>0.05

Fig. 1. Polyacrylamide gel electrophoresis of Eca109 ALP (left sample was heated at 65 ℃ for 10 min prior to electrophoresis)

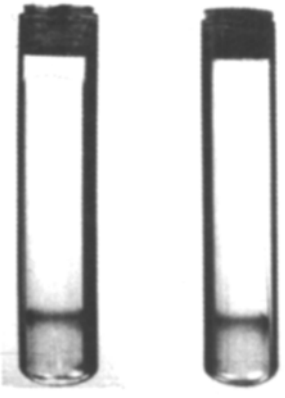

Fig. 2. Polyacrylamide gel electrophoresis of Eca109 ALP after prednisolone treatment (left sample was heated at 65 ℃ for 10 min prior to electrophoresis)

Effect of Prednisolone on LDH of Eca109 Cells

Compared with control cells, the LDH specific activity of cells was no significant change from 12 to 96 h after prednisolone treatment (P>0.05 (Table 2).

DISCUSSION

In humans, there are three main types of ALP isozyme. Encoded by different gene locus, they have some properties distinguished from each other. In the present paper, ALP of Eca109 cells were studied by means of specific inhibition assay and polyacrylamide gel electrophoresis. The enzyme of Eca109 cells was heat stable and was strongly inhibited by L-phenylalanine, but slightly inhibited by urea, it had the same properties as those of PLAP. Furthermore, polyacrylamide gel electrophoresis showed one sharp band of ALP. These results suggested that Eca109 cell was monophenotypic for ALP isozyme which can be classified as PLAP and the enzyme activity could increase naturally during the growth stage.

In normal development, PLAP was expressed at a high level in placenta and a low level in non-malignant cervix and testis.[6] However, non-malignant esophageal tissue has not been found the activity of ALP. PLAP ectopically expressed in Eca109 cells may be involved to derepression of the gene in the malignant process.

It has been reported that ALP activity in some tumor cells could be induce by prednisolone, sodium butyrate and other factors.[7] Hormone induction was found to be specifically restricted to the PLAP. To determine whether prednisolone induction in Eca109 cells is in common with other tumor cells, the cells were treated with prednisolone (1 μg/ml). The induction was observed and the induced ALP was also identified as placental type isozyme. No concomitant increase in LDH activity after prednisolone treatment suggesting that prednisolone could specifically induce increase of PLAP activity in Eca109 cells.

As a biochemical marker of malignant tumor, placental alkaline phosphatase is a effective criterion in the diagnosis, treatment and prognosis of some cancer.[8] This is the first report documenting expression of the enzyme in esophageal cancer cell line. To study the expression and modulation of PLAP further may be useful for the diagnosis and treatment of esophagela tumor.

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