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 a 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 an 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

Cell Culture and Prednisolone Treatment

Eca109 cells were obtained from Department of Cell Biology, Cancer Institute, Chinese Academy of Medical Sciences. Cells were cultured at 37 °C 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 activity was expressed in units per mg protein.

Inhibition Studies

Heat inactivation of ALP was performed by heating samples in a water bath at 65 °C for 10 min. L-phenylalanine or urea inhibition 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. Enzyme activity was visualized by staining the gel according to the method of Lee with disodium α-naphthylphosphate as substrate and diazo fast blue RR as dye.

**Lactic Dehydrogenase (LDH) Activity Assay**

LDH was measured according to the published procedure. 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 °C 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 (%) | P               |
| Heat (65 °C, 10 min)                   |                 |                 |
| Control                               | 0               |                 |
| Prednisolone treated cells            | 0               |                 |
| L-phenylalanine (10 mmol/L)           | 80.83±1.57      | >0.05           |
| Urea (2 mol/L)                        | 25.23±1.12      | >0.05           |

| Table 2. Effect of prednisolone on the activity of ALP and LDH in Eca109 cells (x̄±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   | <0.01          |
| 48                                    | 0.112±0.001      | 0.353±0.002     | <0.01          | 17.175±3.462   | 17.219±2.987   | <0.01          |
| 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          |