EFFECT OF GROWTH IN VIVO AND IN VITRO ON PHENOTYPE OF HEXOKINASE ISOZYMES IN HEPATOMA CELLS

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SUMMARY

Cells of Yoshida ascites hepatoma AH7974 contained type I, II and III hexokinases in vivo during intraperitoneal transplantations in rats. However, culture line of these cells in vitro had only type I and II hexokinases. Type III hexokinase reappeared in addition to types I and II when these culture cells were back-transplanted intraperitoneally into rats. Moreover, when cultured cells were maintained in diffusion chambers in the peritoneal cavities of rats, type III hexokinase appeared again. These observations indicate that cancer phenotypes can be changed by enviromental conditions.

Abnormalities in gene expression in cancer cells have been suggested from studies on isozymes (1-7) and on other phenotypes including cell membrane characteristics (8). We observed a difference in the isozyme patterns of hexokinase in cancer cells and normal cells (9). In a previous paper we also reported that a slow-growing substrain derived from Yoshida sarcoma had type I, II, III and IV hexokinases while the rapidly-growing parent strain had only type I and II hexokinases (9).

This paper reports a difference in the hexokinase isozyme patterns of Yoshida ascites hepatoma AH7974 grown in vivo and in vitro and the instability of cancer phenotypes is discussed.

MATERIALS AND METHODS

Animals and Tumor Cells

A strain of Yoshida ascites hepatoma AH7974 was maintained in male JAR-2 strain rats weighing 80-100 g. A culture line of AH7974 cells (JTC-16) was established from ascitic cells (10). Culture cells were grown at 37°C in TD 40-type glass culture flasks sealed with rubber

stoppers containing 10 ml of Eagle's minimal essential medium (MEM, Nissui Co. Ltd., Tokyo) supplemented with 10 % calf serum, 50 U/ml of penicillin G and 100 ug/ml of streptomycin sulfate. Medium was renewed every 4 days and the cells were subcultured every 10-14 days.

Back-transplantation of culture cells into rats was carried out by intraperitoneal injection of 10^6 cells suspended in 0.1 ml of serum-free Eagle's MEM and 10 to 14 days later ascitic tumor cells were harvested.

Diffusion Chamber Experiments

Millipore filters (25 mm diam., pore size 0.45 u) were attached to both sides of the ring of a diffusion chamber (25 mm diam. x 3 mm) with MF cement (Millipore Filter Corp., Mass.) and the chambers were sterilized with ethylene oxide gas before use. About 10^7 culture cells suspended in 1.0 ml of serum-free Eagle's MEM were injected into the chamber with a syringe. Then the hole in the ring was sealed with sterile cement. Diffusion chambers containing cells were inserted into the peritoneal cavity of rats by operation. After various periods in the peritonal cavities the diffusion chambers were taken out and the cells in the chambers were collected. The number of the cells was counted and their viability was estimated by erythrocin staining. At the same time the hexokinase in the ascitic fluid was analyzed.

Hexokinase Isozyme Analysis and Activity Assay

Cells from culture flasks, the peritoneal cavity of rats or diffusion chambers were washed with cold physiological saline. They were homogenized in the same volume of 0.1 M Tris-HCl buffer (pH 7.4) containing 5 mM EDTA, 5 mM 2-mercaptoethanol and 10 mM glucose using a sonicator (Tomy Co. Ltd., Tokyo). Normal rat liver which had been perfused with cold physiological saline was homogenized in the equal volume of the same buffer in a Potter-Elvehjem type homogenizer. Homogenates were centrifuged at 105,000 x g for 60 min in an ultracentrifuge (Hitachi Ltd., Tokyo) and the pattern of hexokinase isozymes in the supernatant was examined by the method using cellulose acetate membrane electrophoresis (9). Activity was assayed as reported previously (9). Protein was assayed by the method of Lowry et al. (11).

RESULTS

Fig. 1 represents the hexokinase isozyme patterns of normal rat liver and of AH7974 cells after serial transplantation in rats, cultivation for 7 years and growth in the peritoneal cavity of rats after back-transplantation. Transplanted hepatoma cells in vivo showed type I, II and III hexokinases, type II being predominant, as previously reported (9). The culture strain of the hepatoma cells, however, contained only type I and II hexokinases. When these cultured cells were back-transplanted into the peritoneal cavity of rats, types I and II were retained and type III hexokinase reappeared. The specific hexokinase activities of tumor cells

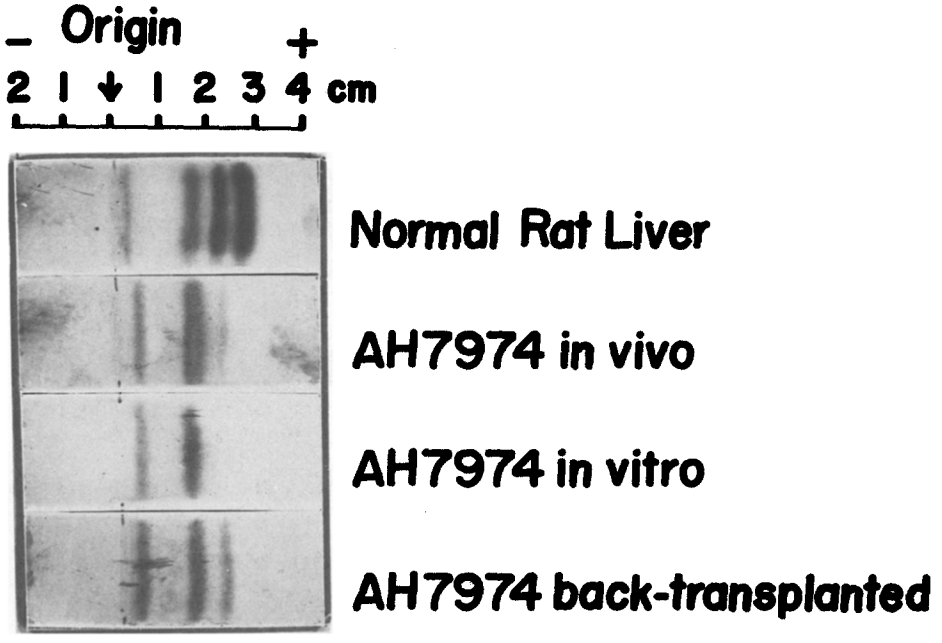

Fig. 1. Hexokinase isozyme patterns on cellulose acetate membrane electrophoresis of normal rat liver, and tumor cells maintained in vivo, cultured in vitro and back-transplanted into rats. The bands (in order of increasing mobility to the anode) are : Type I, II, III and IV.

in the above three conditions were 35-40 units/g protein and no difference was observed among them.

Fig. 2 shows the hexokinase isozyme patterns of cultured cells after growth in diffusion chambers in the peritoneal cavity for various periods. As also shown in Fig. 1, only type I and II hexokinases were present at 0 time. No type III hexokinase was detectable one day after insertion of the diffusion chambers into rats. However, after two days, type III hexokinase was definitely present. After 3 and 4 days, the bands of isozymes including type III on the cellulose acetate membrane were fainter. At these days the activity of hexokinase had also decreased as represented in Table 1. The table shows the number, viability and specific hexokinase activity of cells grown in diffusion chambers for various periods. The total cell number in the diffusion chamber fluctuated considerably and did not increase throughout the experimental period. The cell viability

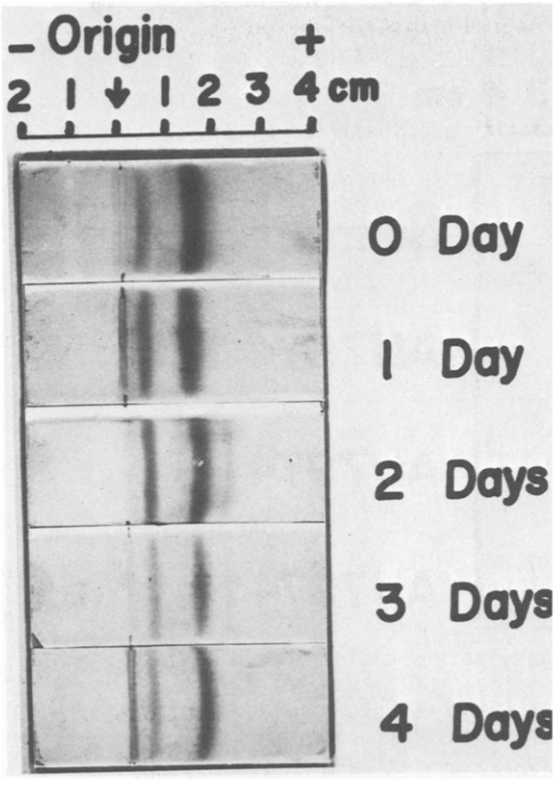

Fig. 2. Hexokinase isozyme patterns of tumor cells in diffusion chambers in the peritoneal cavity of rats.

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and specific activity of hexokinase gradually decreased. The hexokinase activity of ascitic fluid remained less than 0.5 units/g protein and on electrophoresis, bands of hexokinase isozymes were hardly detectable.

DISCUSSION

Many phenotypes of cancer cells were thought to be stable under in vivo and in vitro conditions. But recent findings, including that of loss of transplantability of tumor cells during in vitro cultivation (12) and of the possible maturation of leukemic cells into differentiated cells (13-15) indicate that this is not the case. We also detected differences in the hexokinase and lactate dehydrogenase isozyme patterns of a fast-growing tumor line and slowly-growing substrains derived from it (9,16). Moreover, Bhatnagar and Morgan recently reported that the activity of

Table 1

Cell Number, Viability and Specific Hexokinase Activity of Tumor Cells in Diffusion Chambers[1]

Period Days	Cell Number %	Viability %	Specific Activity units/g protein
0	100.0[2]	75.1	36.4
1	79.1	56.6	36.2
2	107.3	61.3	26.0
3	82.8	38.6	18.0
4	119.7	15.8	20.5

1. Values are averages of 5 to 8 determinations.

2. The cell number at 0 time is expressed as 100 %.

hexokinase, especially of type I was decreased in culture cell strains of mouse tumors which had lost tumorigenecity (17).

These observations indicate that the phenotypic expressions of tumor cells are not constant but that they may change spontaneously or by some modification of enviromental conditions. The present experiments clearly showed that a biochemically detectable phenotype of cancer cells can be changed. It seemed possible that the reappearance of type III hexokinase might be due to the effect of non-tumor cells of the host containing type III. However, this possibility was excluded by results of the experiment using diffusion chambers, since no contamination of the cells in the diffusion chamber with non-tumor cells of the host was observed during the experiment. The gradual decrease in the viability of cells in the diffusion chambers may be due to insufficient fluid transport to support cellu-

lar metabolism, mainly caused by attachment of the peritoneum to the outer surface of the diffusion chamber. The lack of increase in the number of the cells in the chambers is possibly due to the initial high density of these cells. The fact that type III hexokinase appeared in the tumor cells after only 2 days in the diffusion chamber without concomitant increase in cell number suggests that its appearance does not require cell division. Among the four isozymes of hexokinase, activities of type IV (glucokinase) and type II are reported to vary with dietary and hormonal conditions (18, 19) but type III does not seem to be affected by these factors. However, at least in the hepatoma cell line, the presence of type III was very variable. When present, type III hexokinase is always found with types I and II in various tissues and its physiological function is not yet clear.

When hepatoma cells which had been grown in vivo and contained type III hexokinase, were recultivated in vitro, type III gradually disappeared, being weak after 15 weeks and completely absent after 23 weeks (data not shown). This is in contrast to the rapid appearance of type III hexokinase in cells in the diffusion chamber after 2 days. Some in vivo conditions, such as the glucose concentration (20) might be related to the presence of type III hexokinase. Experiments on the reason for the appearance of type III are now being carrid out.

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