Aldolase C in Cultured Mouse Glioblastoma Cells

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SUMMARY

Cells of a transplantable mouse glioblastoma were cultured in vitro. Aldolase C, which is specific to the brain and nerve among normal adult mammalian tissues and was retained in the transplanted tumor in vivo, was also detected in cells after cultivation for more than 200 days. The isozyme pattern of aldolase and the presence of glia cell-specific S-100 protein suggested that these cultured cells were glioblastoma cells. The aldolase isozyme specific to nerve tissues seems to be a useful marker for study of differentiation of glia cells.

INTRODUCTION

There have been many studies on phenotypic expressions of mammalian cells with the use of cells cultured in vitro. In these studies, the choice of cells with suitable phenotypic markers is important. We previously reported on a strain of transplantable glioblastoma from mouse, which retained aldolase C specific to brain tissue (13). Aldolase C is also detected in human brain tumors classified as gliomas, which are of neuroectodermal origin like nerve cells, but this enzyme is not present in brain tumors which are not of neuroectodermal origin (6, 12, 20). This paper reports the presence of aldolase C in cultured cells of the mouse glioblastoma, and the possibility of using aldolase isozyme as a marker of glia cells is discussed together with the use of some other phenotypic markers.

MATERIALS AND METHODS

The mouse glioblastoma was originally induced by methylcholanthrene in the brain of a C57BL strain mouse and was serially transplanted s.c. in the same strain of mice, as described previously (13). At the 37th generation of in vivo transplantation, the tumor was excised 28 days after inoculation, minced, and treated with 0.2% trypsin (Difco Laboratories, Detroit, Mich.) in phosphate-buffered saline (pH 7.2) solution for 15 min at 37°C. The dispersed cells were collected and cultured with an inoculum size of 10⁶ cells/ml at 37°C in TD-15 type of glass culture flasks with rubber stoppers. Each flask contained 2 ml of Eagle’s minimal essential medium (Nissui Co. Ltd., Tokyo, Japan) supplemented with 10% calf serum, 50 units/ml of penicillin G (Takeda Co. Ltd., Osaka, Japan) and 100 µg/ml of streptomycin sulfate (Tyo Co., Ltd., Tokyo, Japan). The medium was renewed every 2 to 3 days. Seven days after inoculation, when the flasks contained confluent cells which adhered to the glass surface, cells were scraped off with a rubber cleaner and 2 X 10⁶ cells were transferred to each of new culture flasks. These cells were continuously cultured for more than 200 days and up to the 10th subculture. For studies on the aldolase isozyme, 10⁶ cells in 10 ml of medium per culture flask were transferred to several TD-40 glass culture flasks with rubber stoppers.

When cells on the glass surface became confluent, the culture medium was discarded and cold phosphate-buffered saline solution was added. Cells were removed mechanically from the glass surface and packed by centrifugation. After sonic disruption at 20 kc for 1 min in a Tomy sonicator, a 50% homogenate in solution of 0.15 M KCl, 1 mM EDTA, and 20 mM Tris-HCl buffer (pH 7.4) was prepared. The homogenate was centrifuged at 105,000 X g for 60 min with a Hitachi ultracentrifuge. The aldolase isozyme in the supernatant fraction was analyzed by cellulose acetate membrane electrophoresis, and activity was measured as previously reported (9). Protein was assayed by the method of Lowry et al. (8). The aldolase isozyme in normal brain of a C57BL mouse and in a s.c. brain tumor were also analyzed.

RESULTS AND DISCUSSION

The aldolase isozyme pattern of cultured tumor cells is shown in Fig. 1, in comparison with those of normal brain and the s.c. tumor. Aldolase C activity was weak but definitely present in cultured cells together with aldolase A and 3 A-C hybrid bands, indicating that the gene for aldolase C peptide was functioning. This pattern of aldolase isozyme was similar to those of normal brain and the s.c. tumor. The aldolase activity of cultured cells for fructose 1,6-diphosphate was 90 to 120 units/g of protein and the ratio of the activities for fructose 1,6-diphosphate to fructose 1-phosphate was 15 to 24.

The aldolase isozyme pattern of cultured cells was kept unchanged throughout the culture period, judging from the results of 4 experiments on aldolase analysis.

Fig. 2 represents the morphological appearance of cultured cells after 180 days of cultivation. At this stage, cells were almost homogenous in morphology, showing relatively small nuclei and many cytoplasmic granules. Cytoplasmic processes in various lengths are exhibited and long ones are attached to other cells forming a network. Active locomotion and movement of these cells were also observed by cinemicroscopy.

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After 90 days of cultivation, 10⁶ cells were inoculated s.c. into 3 C57BL mice. In each case a tumor developed and killed the animal. Morphological identification of the cultured cells with various staining methods has not yet been successful, but analysis of aldolase isozyme revealed the presence of aldolase C, specific to nerve tissues, in these cultured cells. In normal mouse tissues other than nerve and in several transplanted mouse tumors examined, such as hepatomas and mammary tumors, the aldolase isozyme pattern differed from that shown in Fig. 1. Sometimes fetal rat liver and fast-growing neoplastic tissues of rats which originated from tissues other than the nerve possessed aldolase C, suggesting inappropriate phenotypic expressions in cancer cells (14, 15, 19). However, brain tumors are generally slow growing and their histological appearance is similar to that of normal tissues from which they originate. Accordingly, rather benign tumor cells as gliomas may maintain the phenotypes of original normal cells.

S-100 protein is also regarded as a biochemical marker of glia and glioma cells (1, 5, 10). A crude extract of this mouse tumor in vivo was also confirmed to contain a fraction reacting with anti-S-100 protein, as revealed by the immunodiffusion method (11). These observations, together with the fact that only brain tumors of neuroectodermal origin contain aldolase C, strongly suggest the neuroectodermal origin of the cultured cells and that they may possibly be glioblastoma cells. Acetylcholine esterase activity or morphological change has been widely studied in relation to differentiation of neuroblastoma cell lines in vitro (2–4, 16–18). The physiological role of aldolase C, which always coexists with aldolase A (muscle type) in adult nerve tissues, is still obscure. In fetal brain, however, pure aldolase C has not been detected (7, 12). This suggests that the presence of aldolase C is related to differentiation of nerve tissues. Thus, aldolase C may be a marker not only for identification of glia cells but also of their differentiation.

REFERENCES

Fig. 1. Aldolase isozyme patterns of normal mouse brain, s.c. tumor, and cultured tumor cells.
Fig. 2. Phase-contrast photomicrograph of cultured cells on the 180th day of cultivation. X 200.
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