Hydrolytic Enzymes (Acid Phosphatase and Beta-glucuronidase) in Cultured Brain Tumors

Hiroshi Kamitani, Hideaki Masuzawa, Jinichi Sato and Michio Okada*

Abstract

A histochemical study of hydrolytic enzymes such as acid phosphatase and beta-glucuronidase was made on cultured brain tumor cells. Well-differentiated astrocytic cells, oligodendroglioma cells, and medulloblastoma cells showed low activities. Migrating cells and macrophage-like cells showed intense activities. Meningioma cells and schwannoma cells showed relatively intense activities at the early stage of cultivation. At the aged stage, the former showed a characteristic pattern of reaction products. The intensity and distribution of beta-glucuronidase were similar to those of acid phosphatase in cultured brain tumors. The relationship of hydrolytic enzymes to cellular differentiation and viability was discussed.

Key words: brain neoplasms, tissue culture, acid phosphatase, glucuronidase

Introduction

Hydrolytic enzymes, especially acid phosphatase have frequently been studied in tissue sections of brain tumors. Some authors have noted that acid phosphatase activities are associated with regressive changes in brain tumors. Others have suggested a relationship between acid phosphatase activities and cellular ribonucleoprotein synthesis.

For investigations of enzymatic activities of tumor cells, cultured cells may be preferable to tissue sections. Hydrolytic enzymes of cultured brain tumors have rarely been studied. In different cell lines and various stages of cell growth, cells cultured by the monolayer technique were histochemically studied for acid phosphatase and beta-glucuronidase.

Materials and Methods

Tumor tissues were obtained at craniotomy and minced aseptically with scissors into small fragments (1 mm³). Cultivations were made in Earle’s flasks and incubated at 37°C. Exchanges of the nutrient medium, which contained minimum essential medium (GIBCO Laboratories, New York, U.S.A.), fetal bovine serum (GIBCO Laboratories, New York, U.S.A.), and kanamycin (80 μg/ml), were made every five days. Selected cultured cells on cover glasses at various stages of cultivation were rinsed with Dulbecco’s phosphate buffered saline (GIBCO Laboratories, New York, U.S.A.) and fixed in neutralized formal calcium for 30 minutes at room temperature. After washing of fixed cultured cells in distilled water, histochemical reactions were made, following the method of Barka-Anderson, with alpha-naphthyl phosphate and hexazonium pararosanilin at 37°C for 60 minutes. Nuclei were stained with hematoxylin. Histochemical reactions of beta-glucuronidase were also made, based on the method of Hayashi, with naphtol AS-BI beta-glucuronidase and hexazonium pararosanilin at 37°C for 30 minutes. Nuclei were stained with a 1% solution of methyl-green. This study included 4 astrocytomas, 6 glioblastomas, 1 oligodendroglioma, 1 meningioma, and 1 schwannoma.

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Fig. 1  A: Bipolar or multipolar cells showing low acid phosphatase activity. At the early cultivation of astrocytes. ×200. B: Multipolar astrocytes with moderate acid phosphatase activity were conspicuous, and reaction products were distributed peripherally in processes. ×200.

Fig. 2  A: Frequent multipolar astrocytes showing low acid phosphatase activity are seen in cultivation of glioblastoma. ×300. B: Migrating cells with intense acid phosphatase activity and mesenchymal cells with moderate activity are also seen. ×200. C: Multinucleated giant cells showing moderate acid phosphatase activity distributed to the perikaryon. ×200.

Fig. 3  Oligodendroglioma cells showing low acid phosphatase activity. No specific pattern of activity was revealed. ×300.

Fig. 4  Medulloblastoma cells showing low acid phosphatase activity and scattered mesenchymal cells showing moderate activity. ×300.

Fig. 5  A: Migrating cells or microglia-like cells of meningioma showing moderate to intense acid phosphatase activity. ×300. B: At the aged stage, intense and coarsely granular reaction products are seen diffusely in the cytoplasm of meningioma cells. ×300.

Fig. 6  A: Frequent microglia-like cells showing intense acid phosphatase activity at the early cultivation of schwannoma. ×480. B: At the aged stage, schwannoma cells showing low acid phosphatase activity. ×300.
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Fig. 7 Globoid malignant lymphoma cells showing the most intense acid phosphatase activity and fibroblasts showing moderate activity. × 300.

Fig. 8 Mitotic cells showing moderate to intense acid phosphatase activity distributed diffusely in the cytoplasm. × 300.

Fig. 9 Beta-glucuronidase activity in meningioma cells at the aged stage of cultivation. Note the pattern similar to that of acid phosphatase. × 200.

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1 medulloblastoma, 11 meningiomas, 10 schwannomas, and 1 malignant lymphoma.

Results

I. Acid phosphatase

Astrocytoma: At the early stage of cultivation (within 2 weeks), slender cells with bipolar or multipolar processes were frequently observed (Fig. 1A). These cells contained low acid phosphatase activity. At the aged stage (over 2 weeks), moderately activated astrocytic cells with stout processes were conspicuous (Fig. 1B). Astrocytic cells with rich cytoplasm, which were supposed to be well-differentiated, showed little acid phosphatase activity.

Glioblastoma: Cultured cells were composed of various cell shapes. The enzymatic activities of each cell varied. Multipolar astrocytic cells showed low activities (Fig. 2A). On the other hand, large mesenchymal cells showed moderate to intense activities. Migrating cells from explants contained intense reaction products (Fig. 2B). Multinucleated giant cells showed moderate activities which were distributed to the perikaryon (Fig. 2C).

Oligodendroglioma: Cultivated cells had round nuclei and delicate multipolar processes. Some slender cells were seen. Most cells showed low activities. No specific pattern of activity was noted (Fig. 3).

Medulloblastoma: Small, round cells with a narrow rim of cytoplasm were frequent. These cells showed low activities. Among them were scattered large mesenchymal cells with moderate acid phosphatase activity (Fig. 4).

Meningioma: At the early stage of cultivation, migrating cells or microglia-like cells were frequent. These cells showed moderate to intense acid phosphatase activity (Fig. 5A). At the aged stage, a characteristic pattern of acid phosphatase reaction products was revealed. Namely, intense, coarsely granular reaction products were distributed to the perikaryon or diffusely in the cytoplasm (Fig. 5B).

Schwannoma: Like meningioma, at the early stage, microglia-like cells were frequent. Enzymatic activities of these cells were intense (Fig. 6A), and reaction products were distributed diffusely in the cytoplasm. At the aged stage, cultured cells with delicate processes ran in parallel. Enzymatic activities were quite low (Fig. 6B). This pattern was quite different from that of meningioma.

Malignant lymphoma: Cultured cells, including macrophages, showed the most intense acid phosphatase activity among cultured brain tumors (Fig. 7). Reaction products were globoid and distributed diffusely in the cytoplasm.

Mitotic cells: In general, mitotic cells showed moderate activities throughout division. However, mitotic cells of medulloblastoma case showed low acid phosphatase activity. Reaction products of mitotic cells were homogenous and distributed diffusely in the cytoplasm (Fig. 8).

II. Beta-glucuronidase

The intensity and distribution of beta-glucuronidase in cultured brain tumors were generally similar to those of acid phosphatase as shown in a meningioma case (Fig. 9).

Discussion

In our investigations in cultured glioblastomas, various cell shapes were seen, and the acid phosphatase activity of the cells varied. Among these cells, well-differentiated astrocytic cells showed low activities, and no specific pattern of reaction products was revealed. Multinucleated giant cells showed moderate to intense acid phosphatase activity. Similar to well-differentiated astrocytic cells, our cultured isomorphic oligodendroglioma cells showed low activities and no specific pattern of reaction products. Isomorphic oligodendroglioma has been considered to be benign and well-differentiated. These suggest that the activity of acid phosphatase is related to the differentiation of cultured cells. Mitotic cells and spongioblast-like cells contained moderate to intense diffuse acid phosphatase activity in the cytoplasm. On the other hand, multinucleated giant cells, considered to be in regressive change, contained reaction products distributed to the perikaryon. The intensity and distribution of acid phosphatase might be related not only to differentiation but also viability of cultured cells. Despite the high biological viability of medulloblastoma cells, most cultured medulloblastoma cells contained unexpectedly low acid phosphatase activity. Most of the previous reports on medulloblastomas have noted low acid phosphatase activity. However, Duckett et al. reported intense acid phosphatase activity in a fetal medulloblastoma and related it to malignancy.

To date, theories about the origin of malignant lymphoma in the central nervous system have remained controversial. Malignant lymphomas have rarely been cultured. We had an opportunity to culture a malignant lymphoma. Large macrophage-like cells and mesenchymal cells were revealed at the early stage of malignant lymphoma cultivation. These cells showed the most intense acid phosphatase activity among the cultured brain tumors. Of cultured brain
tumors, mesenchymal cells such as meningioma cells and fibroblasts, in general, showed moderate to intense acid phosphatase activity. However, well-differentiated astrocytic cells and oligodendroglia cells showed low activities. From these results, malignant lymphoma could be considered to be of mesenchymal origin.

Fabiani et al., Schiffer et al., and Lolova et al. mentioned low enzymatic activities in tissue sections of meningiomas. Fabiani et al., however, noted intense acid phosphatase activity in meningioma with high cellularity, abundant mitosis, and atypical features. He referred it to ribonucleoprotein synthesis.

Shuttleworth and Allen noted intense acid phosphatase activity in the pia-arachnoid of rat brain. Our investigations of cultured meningiomas revealed a characteristic pattern of acid phosphatase reaction products, namely, intense and coarsely granular reaction products were distributed diffusely in the cytoplasm. Gerhardt et al. noted that, with electrophoretic analysis of acid phosphatase, some meningiomas showed an intense band which was specific to mesenchymal cells. Mitotic cells, in general, can be considered to synthesize DNA actively. Mitotic cells of cultured brain tumors showed moderate to intense acid phosphatase activity that appeared to parallel the amount of cellular DNA. Further investigations should be made to elucidate the relation of hydrolytic enzymes to DNA synthesis. Using a quantitative histochemical technique, Allen investigated enzymatic activities of beta-glucuronidase in brain tumors. He noted that well-differentiated astrocytoma and oligodendroglia showed low activities, whereas glioblastoma showed intense activities. These findings are in accordance with our results. The distribution and intensity of beta-glucuronidase were similar to those of acid phosphatase in cultured brain tumors. This suggested that beta-glucuronidase as well as acid phosphatase might be related to the differentiation and viability of cultured cells.

References

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Address reprint requests to: H. Kamitani, M.D., Department of Neurosurgery, Kanto Teishin Hospital, 5-9-22 Higashigotanda, Shinagawa-ku, Tokyo 141, Japan.