Electron microscopic study of ATPase activity in human brain tumors

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Ultrastructural localization of ATPase was demonstrated in 15 human brain tumors; ATPase activity in the tumor cell was outside the cell membrane and appeared in varying degrees according to the type of tumor. Nonglial tumors such as meningiomas and chromophobe pituitary adenomas showed more intense enzyme activity than gliomas; malignant tumors such as medulloblastoma and glioblastoma multiforme showed low activity. Blood vessels in the tumor showed poor ATPase activity in both endothelium and basement membrane; the lack of ATPase in the vascular wall may contribute to the breakdown of the blood-brain barrier.

KEY WORDS • ATPase • brain tumor • electron microscopy • capillary wall • histochemistry

ADENOSINETRIPHOSPHATASE (ATPase) has been recognized as the energy-dependent membrane transport enzyme and also participates in the regulation of cellular respiration. Bonting, et al.,² have demonstrated general distribution of ATPase in human organs, especially in the nervous system, kidney, retina, intestine, and secretory organs. In the central nervous system, gray matter shows the most prominent activity with less prominent ATPase activity in the white matter, choroid plexus and blood vessels.²,¹²,¹₈ Torack, et al.,¹⁹ using electron microscopy, demonstrated ATPase in the cerebral capillary wall and suggested the significance of ATPase in the basement membrane for transfer regulation in the cerebral capillary.

Laws and O'Connor⁶,¹⁴ have assayed ATPase activity in brain tumors biochemically and histochemically, and have demonstrated that activities are generally less in the tumor than in normal brain tissue. The ultrastructural localization of ATPase in brain tumors has not been reported. This paper reports a study of ultrastructural localization of ATPase activity in 15 human brain tumors.

Materials and Methods

The 15 brain tumors studied included two astrocytomas, two glioblastoma multiforme, one medulloblastoma, two meningiomas, five pituitary adenomas, two neurinomas, and one metastatic tumor.

Tumor tissue obtained at surgery was immediately immersed in a primary fixative made up of 1% paraformaldehyde and 1% glutaraldehyde buffered by cacodylate solu-
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tion. The tumor tissue was cut in small (0.01 mm³) blocks, rinsed in cacodylate buffer, and incubated in the medium of Wachstein-Meisel at 37°C for 60 minutes. The incubated tissue was rinsed in cacodylate buffer, osmicated in 2% OsO₄ solution for 2 hours, dehydrated in graded ethanol and propylene oxide, and embedded in Epon. The sections were cut with an LKB ultramicrotome, double-stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-11-A electron microscope.

In the control group, incubation was done in a medium without ATPase in which the nonspecific reaction of lead was excluded.

Results

The 15 brain tumors were classified into three groups: five gliomas including three malignant tumors, nine nonglial tumors, and one metastatic tumor. ATPase activity was recognized as a small spot or dot indicating lead precipitation. This ultrastructural localization was investigated especially in tumor cells and capillary walls.

Meningioma

The ultrastructure of the tumor cells appeared well preserved even after incubation. There was remarkable ATPase activity in the tumor cells and small vessels (Fig. 1). The activity clearly was localized on the outside of the cell membrane. The metallic deposits that indicate ATPase activity were not seen on the inside of the cell membrane. Fine specific deposits of ATPase were also present in various extracellular spaces and with varying intensity on all the tumor cells. Metal might be deposited over the entire surface of one cell membrane, but on only 70% of the other cell membrane surfaces so that the average distribution was approximately 80%.

Activity was found neither in organelles, such as mitochondria, nor in the nucleus. In the small vessels, corresponding to capillaries, ATPase activity was recognized on the endothelium and basement membranes. Metal was noted on the apposed cell membranes where two endothelial cells contacted each other; there was no deposi-
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Electron micrograph of a meningotheliomatous meningioma showing remarkable ATPase activity (arrow) on the capillary wall localized at the basement membrane (B) and intercellular junction of the endothelial cells (E). There was no activity in the nucleus (N). CL = capillary lumen. X 20,000.

There was no activity in the nucleus (N). CL = capillary lumen.

Pituitary Adenoma

All five pituitary tumors investigated in this study were diagnosed as chromophobe adenomas by light microscopy.

As with meningiomas, the ATPase activity in these adenomas was localized on the outer surface of the tumor cell. No activity specific to the tumor cell, as in secretory granules, was revealed. The nucleus and its membrane or intracellular organelles, such as mitochondria and endoplasmic reticulum, showed no ATPase activity (Fig. 3).

In general, the ATPase activity on the cell membrane was less pronounced than that with meningiomas. The small pituitary vessels, composed of complex endothelium and basement membrane, showed intense ATPase activity at the basement membrane. In general, however, the endothelial ATPase activity was less intense than that with meningiomas. The pericytes showed no activity at all (Fig. 4).

Neurinoma

An acoustic tumor and a spinal cord tumor were studied. The characteristic histological structure of numerous collagen fibrils both inside and outside the cells were seen; the narrow extracellular space showed complicated meshwork.

The ATPase activity in these neurinomas was least often seen among the nonglial tumors. The complicated interdigitated cell membranes showed a relatively intense enzyme activity which was not revealed within the tumor cell (Fig. 5). On the small vessels, slight enzyme activity was noted in the basement membrane; however, the endothelial cell was devoid of enzyme activity.

Astrocytoma

Two astrocytomas diagnosed histologically as benign were examined. The tumor cells...
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Fig. 3. Electron micrograph of a chromophobe adenoma showing moderate ATPase activity (arrow) on the plasma membrane of the tumor cells; no activity in the nucleus (N), nuclear envelope, or organelles. M = mitochondria. X 20,000.

Fig. 4. Electron micrograph of a chromophobe adenoma showing moderate ATPase activity (arrow) in the basement membranes (B) and endothelial cell junctions of the capillary wall. P = plasma membrane, N = nucleus, C = capillary lumen. X 30,000.
characterized by numerous fine gliafibrils were found to contain intense and dense ATPase activity on their cell surfaces. However, the organelles and nucleus showed no enzyme activity (Fig. 6).

The majority of fine vessels in this tumor appeared to have almost normal structure; that is, the basement membrane was of normal thickness and shape. Enzyme activity was localized in the basement membrane slightly. Moreover, the endothelial cells showed little activity on luminal and junctional surfaces (Fig. 7).

Glioblastoma Multiforme

Two glioblastomas showed remarkable degeneration and necrosis, and ultrastructural examination revealed poor preservation. Although assessment of enzyme activity was difficult, slight ATPase activity was noted on the tumor cell.

Within the vascular wall, scattered low enzyme activity could be seen on the basement membrane, even though no enzyme activity was recognized within the cell or endothelial cell.

Medulloblastoma

One of the malignant tumors, a cerebellar medulloblastoma, also showed degeneration and necrosis within the tumor so that ultrastructural localization of the enzyme was difficult. Very little enzyme activity was recognized even on the tumor cell or vascular basement membrane.

Metastatic Tumor

In a metastatic tumor originating from lung cancer, faint ATPase activity could be seen on the tumor cell membrane, and on the basement membrane or the apposed endothelial membranes of the blood vessel. This activity was more intense than that of the other malignant brain tumors studied.

Discussion

Electron microscopic examination of ATPase activity was first described by Wachstein and Meisel,20 and thereafter many investigations have been done on the central nervous system according to their method. Recently, the existence of an
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Fig. 6. Electron micrograph of an astrocytoma showing moderate ATPase activity (arrow) on the plasma membrane of the tumor cell processes, which are filled with gliafibrils (f). X 30,000.

Fig. 7. Electron micrograph of an astrocytoma showing slight ATPase activity (arrow) on the capillary wall in the basement membrane (B). CL = capillary lumen, E = endothelial cell, P = pericyte. N = nucleus. X 15,000.
enzyme that is influenced by $^+$Na and $^+$K ions and by ouabain has been described.\textsuperscript{4,6,11,15} Novikoff, et al.,\textsuperscript{13} and Farquhar and Palade\textsuperscript{9} demonstrated that the ATPase activity revealed by Wachstein and Meisel's method was not influenced by $^+$Na and $^+$K ions nor by ouabain. Skou and Hilberg,\textsuperscript{15} on the contrary, reported that the Na-K-Mg ATPase is identical with the Mg-ATPase and one enzyme could be converted to the other. Koshiba\textsuperscript{7} demonstrated by histochemical electronmicroscopy the presence of Mg-ATPase and Na-K-Mg ATPase in cancer cells and that these two enzymes seem identical; accordingly, it is doubtful that these two enzymes differ from each other. Moreover, the fact that the enzyme demonstrated by Wachstein and Meisel's method appears to concentrate on the cell membrane suggests a correlation with Na-K-ATPase, which is known to participate in active transport at the cell membrane.

Previous investigations\textsuperscript{4,5,10} have demonstrated ATPase activity in normal brain tissue. Becker and his co-workers\textsuperscript{1} reported enzyme activity on the glial cell membrane which was more intense in the astrocyte than in the oligodendrocyte. Fernando and Blunt,\textsuperscript{4} however, showed just the reverse. They also demonstrated Mg-ATPase on unmyelinated axons. The enzyme activity was also demonstrated on Purkinje and granular cells in the cerebellum.

Torack, et al.,\textsuperscript{19} and Becker, et al.,\textsuperscript{1} reported ATPase activity in cerebral blood vessels on both endothelial cells and basement membrane; the former suggested that the presence of enzyme activity in the basement membrane may be important in transfer regulation within cerebral capillaries.

There are a few papers reporting ATPase activity in brain tumors.\textsuperscript{8,16,17} Stavrou\textsuperscript{16} studied various enzymes in experimental brain tumors with a light microscope. Laws and O'Connor\textsuperscript{8} made biochemical assays of ATPase activities in 59 human brain tumors; all tumors showed less activity than that of normal brain tissue, and histological examination of malignant tumors revealed the least activity. O'Connor and Laws\textsuperscript{14} examined the ATPase enzyme activity of the capillary wall in human brain tumors with a light microscope and obtained the interesting result that the higher the malignancy of the tumor, the lower the ATPase activity. Our present study demonstrated a similar tendency. Laws and O'Connor\textsuperscript{8} suggested that the difference in ATPase content in various tumors might reflect differing degrees of vascularity, and that this might be the explanation for the high values in meningiomas. Although histochemical studies are not suitable for quantitative study, our study also emphasizes the high degree of ATPase activity in meningiomas; moreover, meningioma tumor cells showed more intense ATPase activity than that of other tumor cells and in fact more than the blood vessels of meningiomas.

ATPase in the cell has been thought to be the regulator of cellular respiration,\textsuperscript{9} accordingly, the lack of ATPase inevitably causes disturbance of oxidative phosphorylation and a limited supply of ATP in the tumor cell.\textsuperscript{21,22} Thus, disturbed cellular respiration may result in degeneration or necrosis of tumor cells in malignant gliomas.

The fact that the blood vessels of malignant tumors showed little enzyme activity suggests that membrane ATPase participates in the regulation of transport through the capillary wall and thus plays a role in the dynamics of the blood-brain barrier. Torack, et al.,\textsuperscript{19} demonstrated ATPase in the capillary wall of the normal human brain, and suggested that the basement membrane might participate in the blood-brain barrier mechanism. Therefore, a deficiency of ATPase in the vascular wall may contribute to the breakdown of the blood-brain barrier.

Since the least ATPase activity in tumor cells was consistently observed in the most malignant tumors, this type of analysis may serve as another criterion of the malignancy of any specific tumor.

References

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