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ULTRASTRUCTURAL STUDY OF CYTOCHROME OXIDASE IN ONCOCYTOMA

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ABSTRACT

Light, electron microscopic, enzyme histochemical and ultracytochemical studies were performed on specimens of oncocytoma, obtained from an asymptomatic mass in the right parotid gland of a 61 year old Japanese female.

With the light microscope, the diagnosis was benign oncocytoma.

Electron microscopically, the cytoplasm was filled with a vast number of mitochondria, with numerous cristae, some of which contained glycogen particles.

The tumor cells showed a strong activity of histochemically demonstrable cytochrome oxidase.

At ultrastructural level for the demonstration of cytochrome oxidase, using DAB, many of the mitochondria had reaction product, which was restricted only to the cristae and the intracristal space. Also, the glycogen-containing-mitochondria had the enzyme activity on the cristae and in the intracristal space.

The mitochondria of oncocytes could have considerably active function.

These results suggest that oncocytoma which is characterized by numerous mitochondria could be in hyperactive function rather than degeneration.

INTRODUCTION

Oncocytoma of the major salivary gland tumor is a rare, usually benign lesion and is most often seen in the parotid gland. This tumor consists primarily of cells known as oncocyte with an extremely acidophilic granular cytoplasm.

Recently, electron microscopic examinations[4) 12) 13) 17) 26)] of oncocytes in both normal and pathologic tissues have shown that these cells contain numerous mitochondria which undoubtedly are responsible for granules resulting in the marked cytoplasmic eosinophilia seen by light microscopy.

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It still remains undecided whether the presence of abundant mitochondria in oncocytes represents activated function or degenerative process of these cells.

Histochemical studies[9)30)] at the light microscopic level have revealed that oncocytes in the salivary glands and salivary gland tumor have a high level of the enzymes known to be associated with mitochondria.

However, enzyme activity and accurate localization of respiratory chain in the numerous mitochondria of oncocytes have not been studied at ultrastructural level.

More recently, cytochrome oxidase activity came to be demonstrated electronmicroscopically by a new method[24)] based upon Nadi reaction, using the oxidative polymerization of 3, 3'-diaminobenzidine (DAB) to an osmiophilic reaction product. Cytochrome oxidase plays an important role in the biological function of respiration to furnish energy for processes including synthesis of biological materials and osmotic or mechanical work. This enzyme is closely associated with the membrane system of mitochondria and firmly established as a primary enzyme marker for mitochondria.

Therefore, our experiment was undertaken to demonstrate the cytochrome oxidase activity in mitochondria of oncocytoma cells in order to clarify whether the enzyme localization in the mitochondria is similar to that in mitochondria of normal cells.

CASE REPORT

The patient who was a 61 year old female was admitted to Aichi Cancer Center Hospital on May 19, 1971 for a painless lump in the right parotid gland of more than 4 years, which had been gradually increasing in size and attained approximately 4 cm in diameter for 2 or 3 months prior to admittance.

On May 25, 1971, a right superficial lobectomy was done. The specimen was well encapsulated and measured 4.8 by 4.0 by 2 cm, was firm, light tan, and coarsely nodular. The cut surface was lobulated, and glistening brownish red. The final microscopic diagnosis was benign oncocytoma.

The patient recovered uneventfully. She was discharged on June 3, 1971.

MATERIAL AND METHODS

The tumor tissue was obtained from the mass in the right parotid gland by operation.

For light microscopy, the tissue was fixed in buffered formalin at pH 7.2 and embedded in paraffin. The sections were stained with hematoxylin and eosin (H & E), and with phosphotungstic acid hematoxylin (PTAH) to demonstrate mitochondria.

For histochemistry of cytochrome oxidase, thin slices of tumor were fixed

for 6 hrs in cold 2% glutaraldehyde in 0.2 M phosphate buffer at pH 7.2, and left in buffer over night. Frozen sections 8 microns thick were cut with Pearse cold microtome and incubated for 1 hr at 37°C in a medium containing 10 mg DAB (Sigma), 9 ml of 0.15 M phosphate buffer at pH 7.4, catalase (20 μg/ml), and 12 mg of cytocrome c.

As controls, sections were preincubated for 45 min in potassium cyanid solution (1 mg KCN/ml in 0.15 M phosphate buffer at pH 7.2) prior to incubation for demonstrating cytochrome oxidase activity. Some sections were incubated in complete medium containing 10 mg KCN.

For electron microscopic demonstration of cytochrome oxidase activity, pieces of the tumor were fixed for 30 min at 4°C in a mixture of 4% paraformaldehyde-2.5% glutaraldehyde in 0.15 M phosphate buffer at pH 7.4. After being rinsed for 12 hr at 4°C by several changes of 0.15 M phosphate buffer at pH 7.2, the fixed materials were sectioned (25 micron) with a Sorvall tissue sectioner. Then the sections were collected in phosphate buffer solution and incubated for 60 min at 37°C in a medium for demonstration of cytochrome oxidase or in control medium as indicated above. After incubation, the sections were washed thoroughly in phosphate buffer for 60 min, and were postfixed in 2% osmium tetroxide solution for 2 hrs at 4°C. After dehydration with graded alcohols, they were embedded in Epon, cut with a LKB ultrotome, stained with uranium and lead, and examined with a Hitachi 11 B type electron microscope. Some sections were examined without electron staining.

For routine preparation of electron microscopy, the fixation and embedding procedures by omission of incubation for demonstrating cytochrome oxidase with otherwise indentical treatment of tissue as above mentioned were tried.

RESULTS

PTAH stains revealed the cytoplasm of the oncocytes to be filled with numerous small, dark blue to black granules.

Histochemical sections for cytochrome oxidase revealed a population of remarkably stained, reddish brown granules in the tumor cells. The nuclei were not stained.

By conventional electron microscopic examination, the cytoplasm of the tumor cells were characterized by the presence of numerous mitochondria. The majority of mitochondria were oval in shape and measured 1.0 to 4.0 micron in diameter.

Many of them showed the paralled array or closed packing of numerous cristae. Some mitochondria contained prominent deposits of glycogen deilmited by a single membrane, which was not continuous with the limiting membrane. The glycogen in the mitochondria was mostly of the beta type. All mitochondria lacked intramitochondrial dense granules. Dividing mitochondria

were not noted. Aside from mitochondria, the cytoplasm contained appreciable numbers of lysosomes. Other organelles were generally scanty. The surfaces of some tumor cells had microvilli, but secretory granules were not seen as in normal parotid acinar cells.

The ultrastructural findings in thin sections from materials incubated in the medium for cytochrome oxidase activity revealed that the positive osmiophilic reaction product was localized exclusively to the mitochondria. The intensity of the reaction was fairly similar in all cells but slight variations were observed from mitochondria to mitochondria within a given cell. In the stained mitochondria, the reaction product was mostly restricted to the cristae and the intracristal space, whereas no reaction product was observed in the matrix and the outer compartment. Remarkable activity was seen especially in the mitochondria with parallel array or closed packing of numerous cristae. Also, the cristae and intracristal space of glycogen-containing-mitochondria were intensely positive. Beside these reactive mitochondria, some mitochondria had no reaction product.

Organelles other than mitochondria showed no reaction product for the enzyme.

DISCUSSION

Our histochemical and utrastructural findings were in accord with many other[4) 9) 12) 13) 17) 19) 26) 28) 30)] recent reports on oncocytoma.

The appearance of large numbers of mitochondria with parallel array or closed packing of numerous cristae in oncocytoma seems to be based primarily on the resemblance to the abundant mitochondria with numerous cristae seen in metabolically active[18) 19) 25) 29)] tissues.

The present ultracytochemical study clearly indicates that the mitochondria of oncocytes contain cytochrome oxidase on the cristae and in the intracristal space. This finding suggests that the mitochondria of oncocytes belongs to a type of mitochondria in mature cells. In the cytochemical study of differentiating prosobranch spermatids[1)], cytochrome oxidase was reported to be localized in the matrix of their mitochondria in the immature stage, but to appear only on the cristae and in the intracristal space in mature spermatozoa.

Cytochrome oxidase of normal hepatic cells, heart muscle cells and renal tubular[20) 24)] cells had been noted on the cristae, in the intracristal space and outer compartment of mitochondria. There is some difference in the localization of the enzyme activity of the mitochondria between normal cells and oncocytes. This may represent that the molecular structure of the outer compartment of mitochondria in oncocytes is somewhat different from that of normal cells.

The histochemical[9)30)] and biochemical[23)] tests of oncocytes have been performed in previous studies. There was, however, a discrepancy in the results obtained from the two techniques[26)]. Tandler *et al.* (1970)[26)] explained that the discrepancy in enzyme activity by histochemical and biochemical studies could be due to the marked fragility of mitochondria so that they were easily damaged during isolation. The altered molecular structure in the outer compartment of mitochondria in oncocytes above mentioned may be responsible for this fragility.

The presence of glycogen particles in the mitochondria of oncocytes has been described by previous electron microscopic studies[12)26)]. Intramitochondrial glycogen has been reported to appear also in spermatozoa[1)3)] (Anderson, 1970 and 1968), cells of the silkworm prothoracid gland[5)] (Beaulation, 1964), lumbar spinal ganglia of the frog[6)] (Berthold, 1966), Drosophila heart with age[7)] (Burch *et al.*, 1970), myopathic muscle[8)] (D'Agostino *et al.*, 1968), heart of idiopathic cardiomyopathology[14)] (Hug *et al.*, 1967), rat retinal receptor cells[16)] (Ishikawa *et al.*, 1965), flight muscle from aging blowflies[21)] (Sacktor *et al.*, 1972), nerve cells in bilirubin encephalopathy[22)] (Schutta *et al.*, 1970) and mouse hepatic cells in riboflavin deficiency[27)] (Tandler *et al.*, 1968).

The role of intramitochondrial glycogen is not really understood at present. However, in the ultrastructural study of the spermatozoa, Anderson[2)] described by simultaneous demonstration of the enzyme activity in the mitochondria that intramitochondrial glycogen could be related to energy-production and contribute to the survival of spermatozoa under unfavorable conditions. Sacktor[21)] reported in a study of mitochondria of the flight muscle from aging blowflies that cytochrome oxidase was not evident in the part of mitochondria undergoing morphological change such as glycogen deposition, and the degradation of mitochondria was correlated with decrease in biochemical function. Our interesting finding that respiratory enzyme activity was evident in the glycogen-containing-mitochondria may indicate intramitochondrial glycogen to play some important roles of metabolism in oncocytoma.

The present structural study and cytochemical domonstration of cytochrome oxidase in numerous mitochondria of oncocytes may be interpreted as a mechanism to provide energy production for this type of tumor cells.

From the above, it is quite possible that the oncocytoma cells have some kind of hyperactive function rather than degenerative metaplasia as was stated by Hamperl[10)] (1962).

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EXPLANATION OF FIGURES

FIG. 1. Tumor cells, which are tall columnar to pyramidal in form, have eosinophilic granular cytoplasm (H.E.) (×298).

FIG. 2. The cytoplasm of the oncocyte is filled with numerous mitochondria, which are reactive to cytochrome oxidase. The osmiophilic reaction product is shown on the cristae and the intracristal space (cytochemical E.M.) (×8,000).

FIG. 3. Same tissue as in Fig. 2 at higher magnification. The typical ovoid mitochondrion shows the close packing of the numerous cristae. The reaction product appears only on the cristae and in the intracristal space, while the matrix and the outercompartment are entirely unreactive (cytochemical E.M.) (×54,000).

FIG. 4. An enlarged mitochondrion containing beta-glycogen. The glycogen deposit is delimited by a single membrane, which is not continuous with the limiting membrane of organelle (E.M.) (×27,000).

FIG. 5. The glycogen-containing-mitochondrion has osmiophilic reaction product on the cristae and in the intracristal space (cytochemical E.M.) (×37,5000).

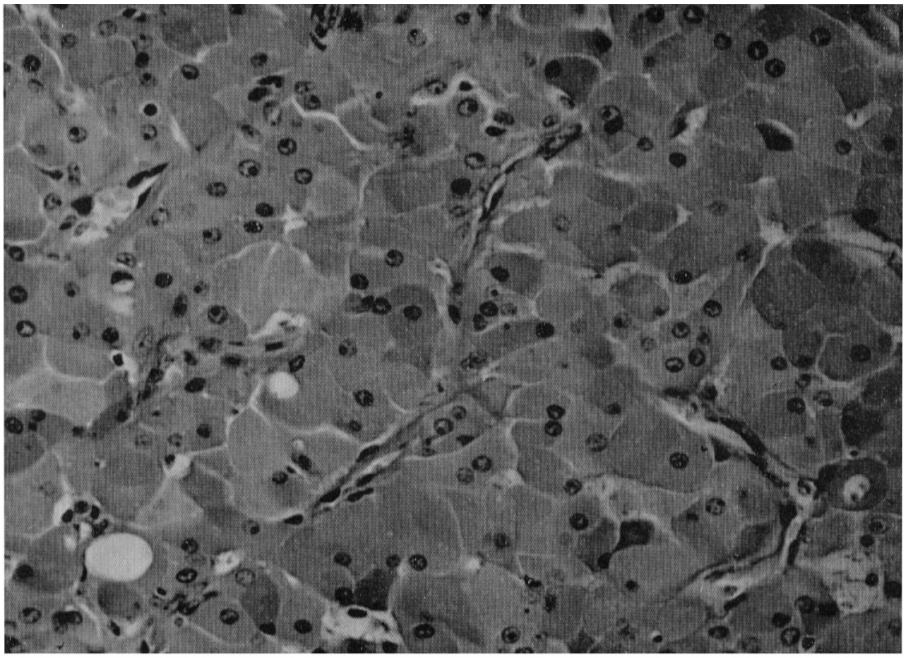

FIG. 1

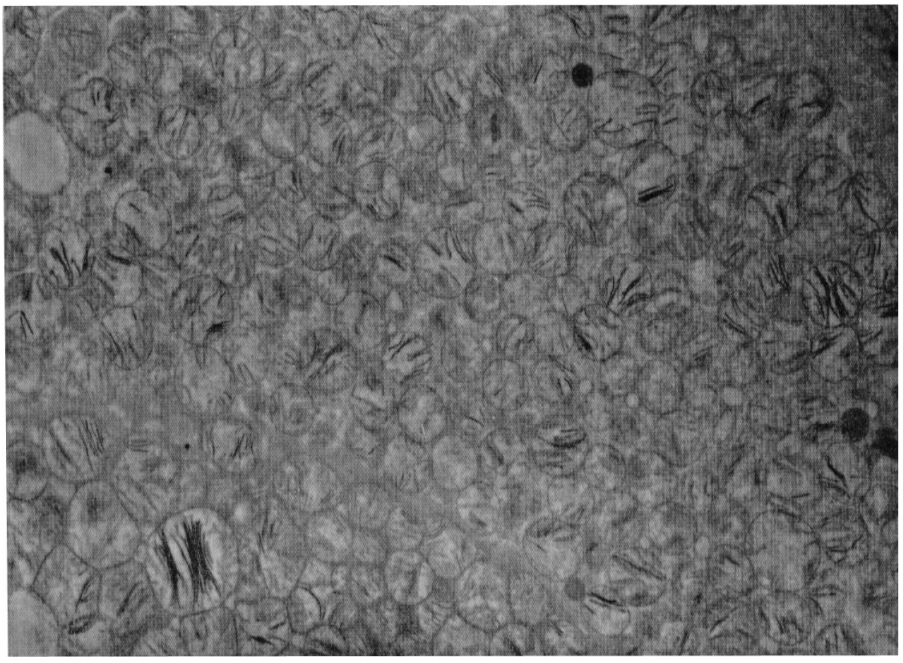

FIG. 2

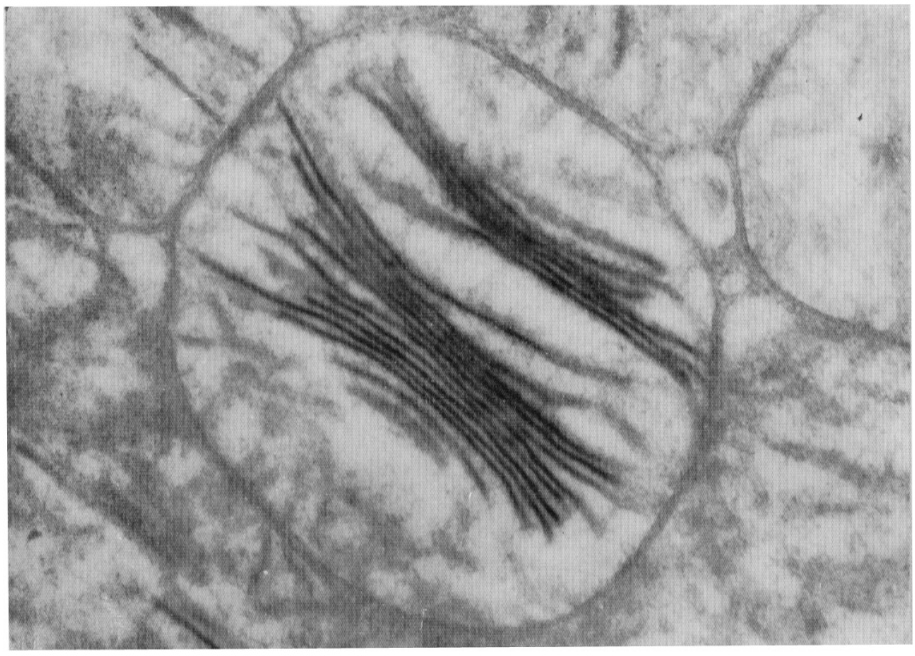

FIG. 3

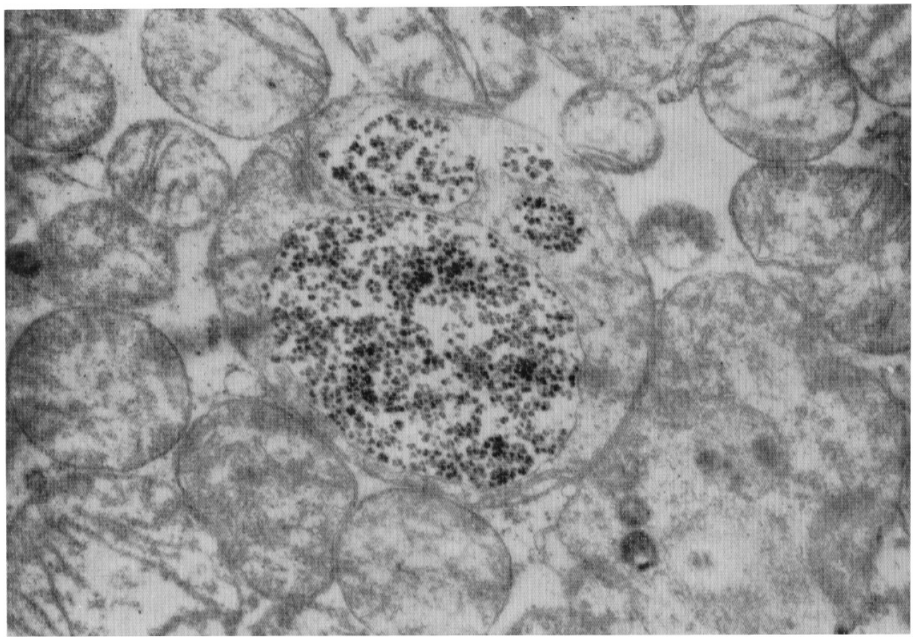

FIG. 4

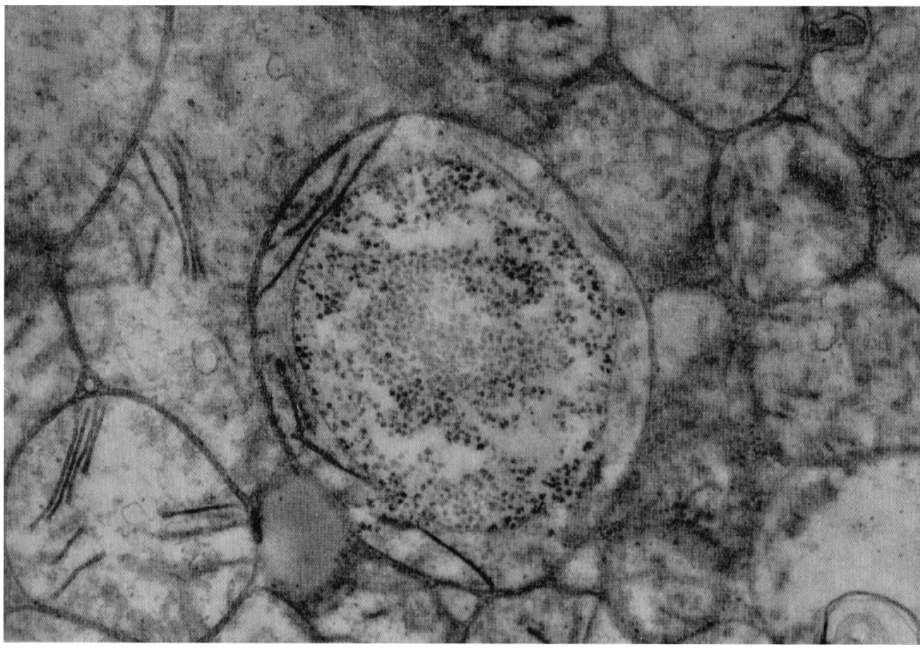

FIG. 5