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Metabolic Activity of Cells in the Macula Flava of the Human Vocal Fold From the Aspect of Mitochondrial Microstructure

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Objectives: There is growing evidence to suggest that the cells in the maculae flavae of the vocal fold mucosa are tissue stem cells of the human vocal fold. This study investigated the metabolic activity of the cells in the maculae flavae of the human vocal fold from the aspect of mitochondrial microstructure.

Study Design: Histologic analysis of the human vocal folds.

Methods: Five normal human adult vocal folds obtained from autopsy cases were investigated under transmission electron microscopy.

Results: Mitochondria were randomly distributed in the cytoplasm of the cells. The morphological features of the mitochondria consisted of a double-membrane-bounded body containing matrices and a system of cristae. In each mitochondria, the lamellar cristae were sparse. The intercristal space was occupied by a mitochondrial matrix which contained electron-dense matrix granules, mitochondrial DNA, and ribonucleoprotein granules. Some mitochondria spread out over or fused to the surface of a lipid droplet in the cytoplasm. In addition, both the mitochondrial outer and inner membranes and the membranes of the lipid droplets had disappeared. Some close association between mitochondria and rough endoplasmic reticulum was present.

Conclusions: The features of the mitochondria of the cells in the maculae flavae of the human vocal fold suggested that their metabolic activity and oxidative phosphorylation were low and that they may have shifted to the utilization of lipids to some extent for their metabolic needs.

Key Words: Metabolic activity, mitochondria, oxidative phosphorylation, beta-oxidation, tissue stem cells, human vocal fold mucosa, macula flava, larynx.

Level of Evidence: NA

INTRODUCTION

As described in detail previously, human adult maculae flavae located at both ends of the lamina propria of the vocal fold mucosa are dense masses of cells and extracellular matrices.[1,2] The histological structure of the maculae flavae in the lamina propria of the human vocal fold mucosa is unique and their roles in the vocal fold as a vibrating tissue are important.[1,2]

Physiologically, the latest research shows the maculae flavae located at both ends of the lamina propria of the human vocal fold mucosa are involved in the metabolism of extracellular matrices, which are essential for the viscoelastic properties of the human vocal fold mucosa as a vibrating tissue, and they are responsible for maintaining the characteristic layered structure of the human vocal fold mucosa.[1,2] Furthermore, human maculae flavae are considered to be an important structure in the growth, development, and aging of the human vocal fold mucosa.[3–5]

The latest research shows there is growing evidence to suggest that the cells in the maculae flavae located at both ends of the lamina propria of the vocal fold mucosa are tissue stem cells of the human vocal fold and the macula flavae are a candidate for a stem cell niche.[6–13]

The mitochondria, one of the intracellular organelles where many vital metabolic reactions take place, is the major source of cellular adenosine triphosphate (ATP) and supplies the cell with most of its usable energy. In general, the number of mitochondria per cell and the number of cristae per mitochondrion are related to the energy requirements for the function carried out by that cell type.[14]

The purpose of this study is to investigate the metabolic activity of the cells in the maculae flavae of the human vocal fold from the aspect of mitochondrial microstructure.

MATERIALS AND METHODS

Five normal human adult vocal folds obtained from autopsy cases ranging in age from 32 to 51 years (mean ± SD, 42.2 ± 7.1 years) were investigated.

The mitochondria in the cytoplasm of the cells in the maculae flavae of the human adult vocal fold mucosa were observed using transmission electron microscopy (TEM).

For TEM, the specimens were fixed in 2.5% glutaraldehyde at 4°C for 2 hours, rinsed with cacodylate buffer solution and postfixed

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in 2% osmium tetroxide with cacodylate buffer solution at 4°C for 2 hours. After rinsing with cacodylate buffer solution, the specimens were dehydrated in graded concentrations of ethanol and embedded in epoxy resin. Semithin sections were prepared with an ultramicrotome, stained with 1% toluidine blue, and examined with a light microscope. Thin sections were made with an ultramicrotome. Thin sections were stained with uranyl acetate and lead citrate. Observation was conducted with a H-7650 (HITACHI, Japan) transmission electron microscope.

To assess the number of mitochondria per cell in the maculae flavae, their numbers were calculated in 50 random cells in the electron micrographs of the specimens. The size of each mitochondrion was measured in each of the 50 cells. To evaluate the concentration of cristae in each mitochondrion, the ratio of cristal space to intercristal and cristal space of the mitochondrion was measured with computer software (ImageJ, NIH, USA) in each mitochondrion.

RESULTS

Mitochondrial Morphology of the Cells in the Human Adult Maculae Flavae

Mitochondria were randomly distributed in the cytoplasm of the cells in the human maculae flavae. Their shape was oval (Fig. 1) and 531–850 nm (579 ± 109 nm, average ± SD) in diameter. Their numbers per cell ranged from 0 to 5 (2.1 ± 1.7) in the electron micrographs of the specimens.

The mitochondria consisted of a double-membrane-bounded body containing matrices and a system of cristae. Mitochondria were limited by smooth-countered outer and inner membranes (Fig. 1). The inner membranes ran parallel to the outer membranes. Cristae, the inner membrane forming thin folds which projected into the interior of the mitochondria (lamellar cristae), were observed (Fig. 1).

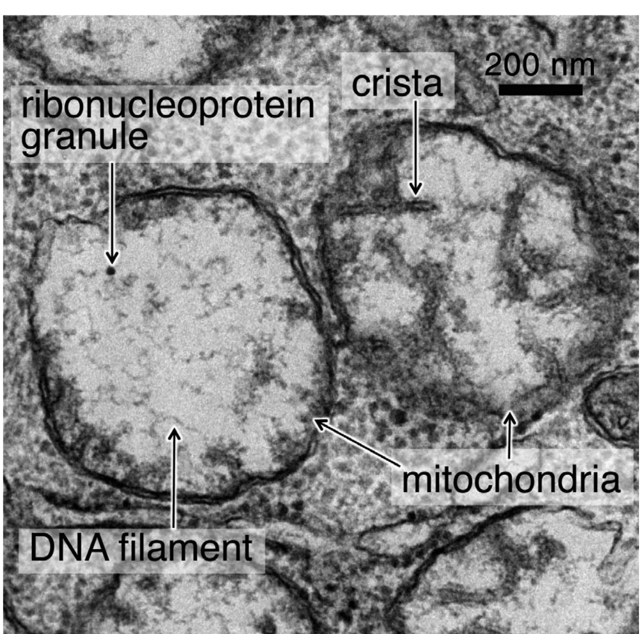

Fig. 1. Mitochondria in the cytoplasm of the cells in the maculae flavae of the human vocal fold (transmission electron micrograph, uranyl acetate, and lead citrate stain). The mitochondrial cristae of the cells in the human maculae flavae were sparse.

The mitochondrial membranes formed two compartments. One was a large intercristal space consisting of all of the area within the inner membrane. Another was a smaller membrane space (outer chamber) comprising of the narrow cleft between the outer and inner membranes and extending inward between the membranes of cristae.

The intercristal space was occupied by a mitochondrial matrix which was as electron-dense as the contents of the membrane space (outer chamber). The matrix contained some electron-dense granules (intramitochondrial granules, matrix granules) 20–40 nm in diameter. Mitochondrial DNA was observed as a lose aggregation of slender filaments in the electron-lucent area of the mitochondrial matrix (Fig. 1). Ribonucleoprotein granules, approximately 12 nm in diameter, were distributed throughout the matrix (Fig. 1).

The transmission electron micrographs showed mitochondrial profiles suggesting impending division or fusion (Fig. 2). The static electron micrographs could not on their own indicate the direction in which a process was moving.

Mitochondrial inclusions were observed in the mitochondrial matrix (Fig. 2).

Concentration of Cristae in the Mitochondrion of the Cells in the Human Adult Maculae Flavae

The mitochondrial cristae were seen as a system of membranous laminae or plate-like structures lying in the mitochondrion. They arose from the inner membrane and transversed a variable distance across the organelle.

The ratio of cristal space to intercristal and cristal space of the mitochondria was 3.4 ± 1.8% (average ± SD). Hence, the characteristic feature of the mitochondrial cristae of the cells in the human maculae flavae was that they were sparse (Fig. 2).

Mitochondrial Associations of the Cells in the Human Adult Maculae Flavae

Close association between mitochondria and lipid droplets in the cytoplasm of the cells in the human maculae flavae was observed. Some mitochondria spread out over or fused to the surface of a lipid droplet in the cytoplasm (Fig. 3A). In addition, both the mitochondrial outer and inner membranes adjacent to the membranes of the lipid droplets had disappeared (Fig. 3B).

Some close association between mitochondria and rough endoplasmic reticulum was present (Fig. 4). Curved profiles of rough endoplasmic reticulum with cisternae, which were moderately distended with proteinaceous secretary product, wrapped around mitochondria (Fig. 4). The rough endoplasmic reticulum partially or almost completely encircled the mitochondria but with a narrow zone of cytoplasm persisting between them. Occasionally, the rough endoplasmic reticulum fused with the mitochondrial membrane (Fig. 4).

DISCUSSION

Mitochondria are intracellular organelles distributed in the cytoplasm of the cells. In living cells, these organelles

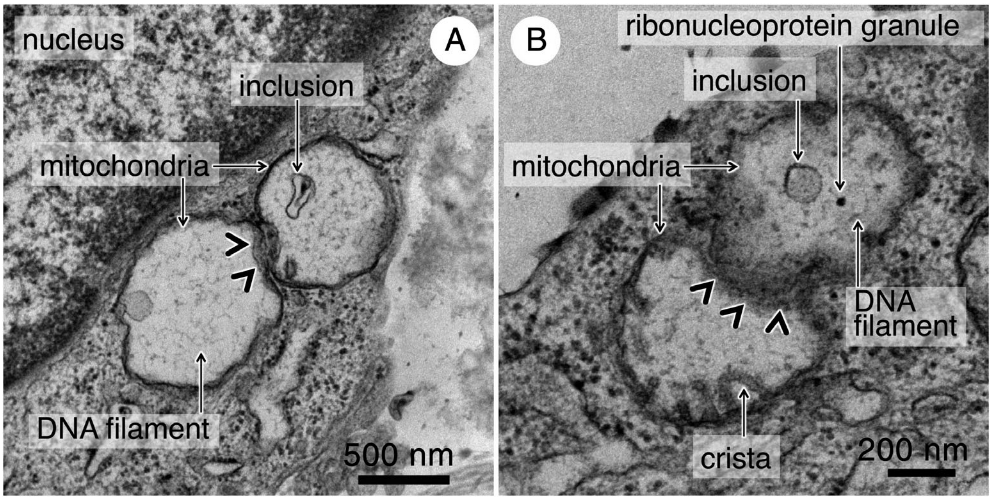

Fig. 2. Impending division or fusion of the mitochondria in the cytoplasm of the cells in the maculae flavae of the human vocal fold (transmission electron micrograph, uranyl acetate, and lead citrate stain). Each mitochondrial outer and inner membrane adjacent to the membrane of another mitochondrion (A: arrowhead) had disappeared (B: arrowhead).

are seen to make slow sinuous movement accompanied by changes in size and shape.[15] Mitochondria show many variations in size, shape, and fine structure; however, they are sufficiently characteristic to be distinguishable from other organelles in electron micrographs.[15]

It is well established that the mitochondria are the major source of cellular ATP.[15] Since the mitochondria supply the cell with most of its usable energy, it is considered to be the powerhouse or powerplant of the cell.[15]

In general, the number of mitochondria per cell and the number of cristae per mitochondrion are related to the energy requirement for the function carried out by that cell type.[14]

The latest research shows there is growing evidence to suggest that the cells in the maculae flavae, located at both ends of the lamina propria of the human vocal fold mucosa, are tissue stem cells of the human vocal fold.[6–13]

In this study, metabolic activity of the cells in the maculae flavae of the human vocal fold was invested from the aspect of mitochondrial microstructure.

General Mitochondrial Morphology of the Cells

In general, the main morphological features can be summarized by defining a mitochondrion as a double-membrane-bounded body containing matrices and a system of cristae.[15] Although the basic morphology of the mitochondrion is very characteristic, innumerable variations occur. Such variations can be considered in terms of species differences, tissue or organ differences, differences in physiological and functional activity, and differences in pathological states.[15]

Most of the tricarboxylic acid cycle enzymes are located in the matrices, and electron transport and oxidative phosphorylation enzymes form molecular assemblies

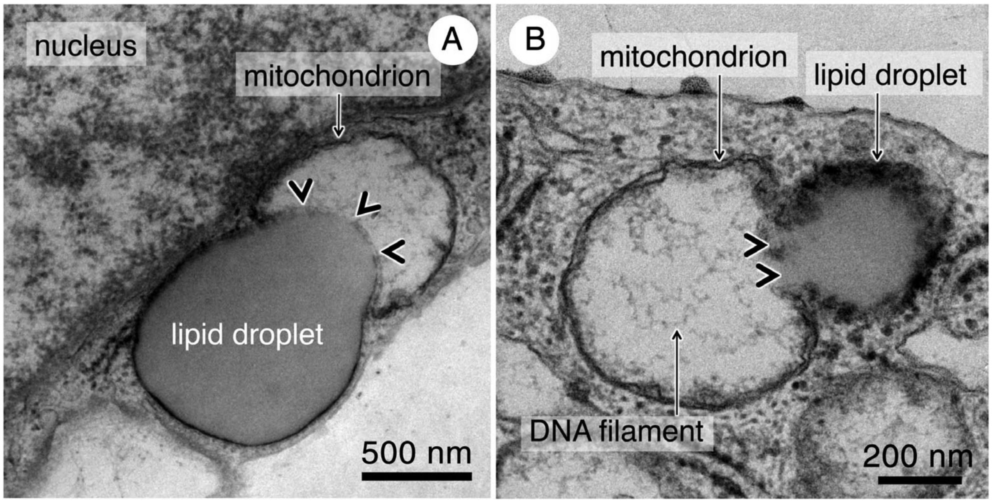

Fig. 3. Close association between mitochondria and lipid droplets in the cytoplasm of the cells in the human maculae flavae. (A) A single mitochondrion spread out over and fused to the surface of a lipid droplet in the cytoplasm (arrowhead). (B) Both the mitochondrial outer and inner membranes adjacent to the membrane of the lipid droplet had disappeared (arrowhead).

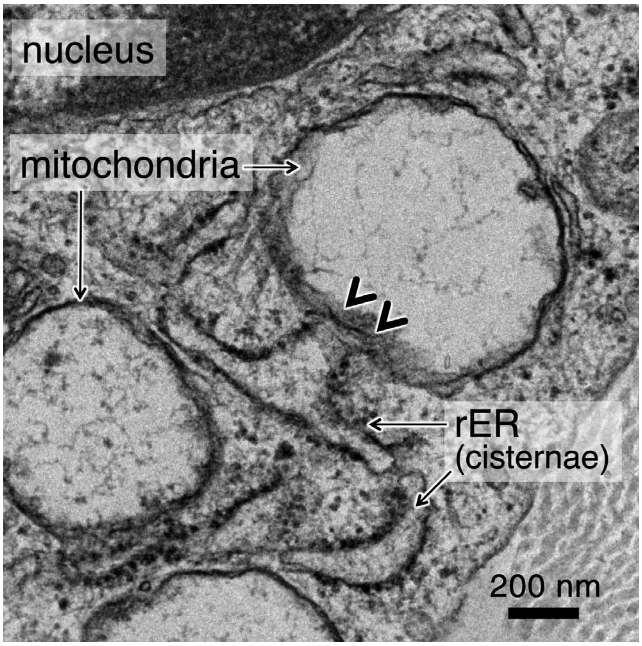

Fig. 4. Close association between mitochondria and rough endoplasmic reticulum. The curved profiles of rough endoplasmic reticulum with cisternae wrapped around mitochondria. The rough endoplasmic reticulum fused with the mitochondrial membrane (arrowhead). rER = rough endoplasmic reticulum.

in or on the inner mitochondrial membrane covering the wall and cristae.[15] The inner membrane and its spheres are thought to be the site of oxidative phosphorylation.[15]

Mitochondrial Morphology of the Cells in the Maculae Flavae of the Human Vocal Fold

The main morphological features of the mitochondria of the cells in the maculae flavae of the human vocal fold were double-membrane-bounded bodies containing matrices and a system of cristae.

There is evidence that mitochondria can divide and multiply and it would appear that old, effete, or damaged mitochondria can suffer regressive changes and be removed in various ways in the cells.[15] The electron micrographs in this study showed mitochondrial profiles suggesting impending division or fusion in the cells in the human maculae flavae. Although the static electron micrographs could not indicate the direction in which a process was moving, these microstructural features suggest that the mitochondria can divide and multiply in the cytoplasm of the cells in the human maculae flavae.

Concentration of Cristae in the Mitochondria of the Cells in the Human Maculae Flavae

The inner membrane forming thin folds that project into the interior of the mitochondria is called the cristae.[15] This plication of the inner membrane is a device for increasing the area of this enzyme-rich membrane.[15]

There is a positive correlation between the metabolic activity of a tissue and the number and size of mitochondria and also the number, size, surface area, and concentration of cristae.[15] The number of cristae per mitochondrion is much greater in cells with high-energy requirements than in those having a lower rate of metabolism.[16]

This point of view suggests that the metabolic activity and oxidative phosphorylation of the cells in the human maculae flavae were low because the mitochondrial cristae were sparse.

Mitochondrial Associations of the Cells in the Human Adult Maculae Flavae

A close association between mitochondria and other organelles seems to be quite meaningful and provides correlation between structure and function. Mitochondria are often located near a supply of substrate or at sites in the cell known to require the ATP generated by the mitochondria.[15]

The human maculae flavae contain vocal fold stellate cells that are stellate in shape and possess lipid droplets in their cytoplasm.[2,17] In addition, the lipid droplets store vitamin A that is an essential liposoluble vitamin.[2,18] The roles of lipid droplets in the cytoplasm of vocal fold stellate cells have been unclear.

In this study, some mitochondria were close to, spread out over, or fused to the surface of a lipid droplet in the cytoplasm of the cells in the human maculae flavae. In addition, both the mitochondrial outer and inner membranes adjacent to the membrane of lipid droplets had disappeared. These microstructural features suggested that the lipid droplets in the cytoplasm of the cells in the maculae flavae supplied the fatty acid degraded by beta-oxidation in the mitochondria. Since the mitochondria are known to contain many of the enzymes (fatty acid oxidases) necessary for the metabolism of triglycerides,[15] these microstructural features also suggested that this brings the mitochondrial enzymes into close association with the lipidic substrate. The cells in the maculae flavae may have shifted to the utilization of lipids to some extent for their metabolic needs.

Another close association in this study was that seen between the mitochondrion and the rough endoplasmic reticulum. Curved profiles of rough endoplasmic reticulum with cisternae wrapped around mitochondria and occasionally, the rough endoplasmic reticulum fused with the mitochondrial membrane. These microstructural features suggested that the mitochondria provide energy for the rough endoplasmic reticulum.

Metabolic Activity of the Cells in the Human Maculae Flavae

The major source of DNA damage in stem cells besides that associated with cell proliferation is endogenously generated genotoxic agents produced as signaling molecules or metabolic byproducts, among which reactive oxygen species (ROS) are the most common threat.[19] Oxidative stress shortens the life span of stem and progenitor cells.[20] ROS accelerate aging through random and sequential damage to cell components.[20]

ROS are continuously generated by normal metabolic processes such as oxidative phosphorylation.[19] The inner mitochondrial membrane covering the wall and cristae is thought to be the site of oxidative phosphorylation.[15] The oxidative phosphorylation in the mitochondria is the major source of endogenous ROS.[19] There is usually a good correlation between the metabolic rate and the level of ROS generated by mitochondria.[20]

ROS, the most significant mutagens in stem cells, when elevated activate the protective mechanisms blocking self-renewal of the stem cells and at the same time serve as a signal stimulating stem cell differentiation.[19]

Quiescence (G_0 phase) is critical for protecting the stem cell compartment.[20] The quiescent state is generally viewed as a mechanism for avoiding accumulation of damage resulting from physiological stress including oxidative stress.[21] Most of the cells in the maculae flavae of the vocal fold mucosa do not express Ki-67, indicating that they are resting cells (G_0 phase).[11,12] Consequently, the cells in the maculae flavae are suggested to avoid accumulation of damage resulting from oxidative stress.

In this study, microstructural features of the mitochondria suggested that the metabolic activity and oxidative phosphorylation of the cells in the human maculae flavae were low indicating the intracellular ROS production is suppressed. The cells in the human maculae flavae seem to rely more on glycolysis for energy supply in comparison with oxidative phosphorylation. In addition, the cells in the maculae flavae may have shifted to the utilization of lipids to some extent for their metabolic needs.

The metabolism of the cells in the human maculae flavae seems to be favorable to maintain the stemness and undifferentiated states of the cells. Further investigations using other experimental methods are needed regarding the metabolic activity of the cells in the maculae flavae of the human vocal fold mucosa.

CONCLUSIONS

In this study, the microstructural features of the mitochondria of the cells in the maculae flavae of the human vocal fold suggested that the metabolic activity and oxidative phosphorylation were low and may have shifted to other metabolic systems such as the utilization of lipid to some extent for their metabolic needs.

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