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ORIGINAL RESEARCH

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Glycolytic activity of the tissue stem cells in the macula flava of the human vocal fold

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Abstract

Objectives: In our previous studies, the features of the mitochondria of tissue stem cells in the maculae flavae of the human vocal fold suggested that their metabolic activity and oxidative phosphorylation was low. This study investigated the metabolic activity, especially glycolysis of the tissue stem cells in the maculae flavae of the human adult vocal fold.

Study Design: Histologic analysis of the human vocal folds.

Methods: Three normal human adult vocal folds obtained from autopsy cases were investigated using immunohistochemistry.

Results: Among the three phenotypes of cells in the human adult maculae flavae, the vocal fold stellate cell-like cells strongly expressed glucose transporter-1. Three phenotypes of cells in the human adult maculae flavae expressed glycolytic enzymes (hexokinase II, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase A) indicating the tissue stem cells in the maculae flavae relied more on glycolysis. The cells did not express phosphofructokinase-1 but did express glucose-6-phosphate dehydrogenase indicating the cells relied more on the pentose phosphate pathway. The cells expressed lactate dehydrogenase A indicating the maculae flavae of the human adult vocal fold was likely to be an anaerobic microenvironment.

Conclusions: The present study is consistent with the hypothesis that the tissue stem cells in the maculae flavae of the human vocal fold seem to rely more on anaerobic glycolysis, especially by the pentose phosphate pathway, for energy supply. The metabolism of the tissue stem cells in the maculae flavae of the human adult vocal fold is likely to prevent toxic reaction oxygen species and be favorable to maintaining the stemness and undifferentiated states of the tissue stem cells in the stem cells system.

Level of Evidence: NA.

KEYWORDS

glycolysis, human vocal fold mucosa, larynx, macula flava, metabolic activity, oxidative phosphorylation, tissue stem cells

1 | INTRODUCTION

The latest research shows there is growing evidence 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 and the macula flavae are a stem cell niche which is a microenvironment nurturing the stem cells.[1-12]

Tissue stem cells in the maculae flavae of the human vocal fold mucosa are likely 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.[13,14] Hence, tissue stem cells in the maculae flavae of the human vocal fold mucosa are likely to contribute to the growth, development and aging of the human vocal fold mucosa as a vibrating tissue.[15-17]

In order to maintain the stemness and undifferentiated states in the stem cell system, the metabolism of the cells is essential. Our previous research investigated the metabolic activity of the cells in the maculae flavae of the human vocal fold from the aspect of mitochondrial microstructure.[9] Consequently, the cells in the human maculae flavae seem to rely more on glycolysis for energy supply in comparison with oxidative phosphorylation.[9] And the metabolism of the cells in the human maculae flavae seems to be favorable to maintaining the stemness and undifferentiated states of the cells.[9]

The purpose of this study is to investigate the metabolic activity, especially glycolytic activity, of the tissue stem cells in the maculae flavae of the human adult vocal fold.

2 | MATERIALS AND METHODS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on human experimentation (Kurume University) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from the subjects after the nature of the experimental procedure was explained.

Three normal human adult vocal folds obtained from autopsy cases were investigated. Any diseases that could possibly affect the tissue of the vocal fold were not observed.

The cells in the maculae flavae of the human adult vocal fold were observed using light microscopy including immunohistochemistry.

2.1 | Light microscopy (immunohistochemistry)

For light microscopy, specimens were fixed in 10% formalin, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Hematoxylin-Eosin stain was used for each section, and immunohistochemical staining was carried out.

Glucose transporter-1 (GLUT-1), and glycolytic enzymes (hexokinase II (HK II), phosphofructokinase-1 (PFK-1), glucose-6-phosphate dehydrogenase (G6PD), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and lactate dehydrogenase A (LDHA)) (Figure 1) were detected histologically in formalin-fixed and paraffin-embedded tissue by immunohistochemistry, for which a universal immuno-enzyme polymer method staining kit (Histofine Simple Stain MAX-PO, Nichirei, Tokyo, Japan) was used.

A 1:250 antibody against GLUT1 (Abcam, Cambridge, UK, ab115730, rabbit monoclonal), a 1:200 antibody against HK II (Abcam, Cambridge, UK, ab104836, mouse monoclonal), a 1:50 antibody against PFKFB3 (Abcam, Cambridge, UK, ab181861, rabbit monoclonal), a 1:50 antibody against G6PD (Abcam, Cambridge, UK, ab106810, goat polyclonal), 1:50 antibody against GAPDH (Abcam, Cambridge, UK, ab9485, rabbit polyclonal) and 1:250

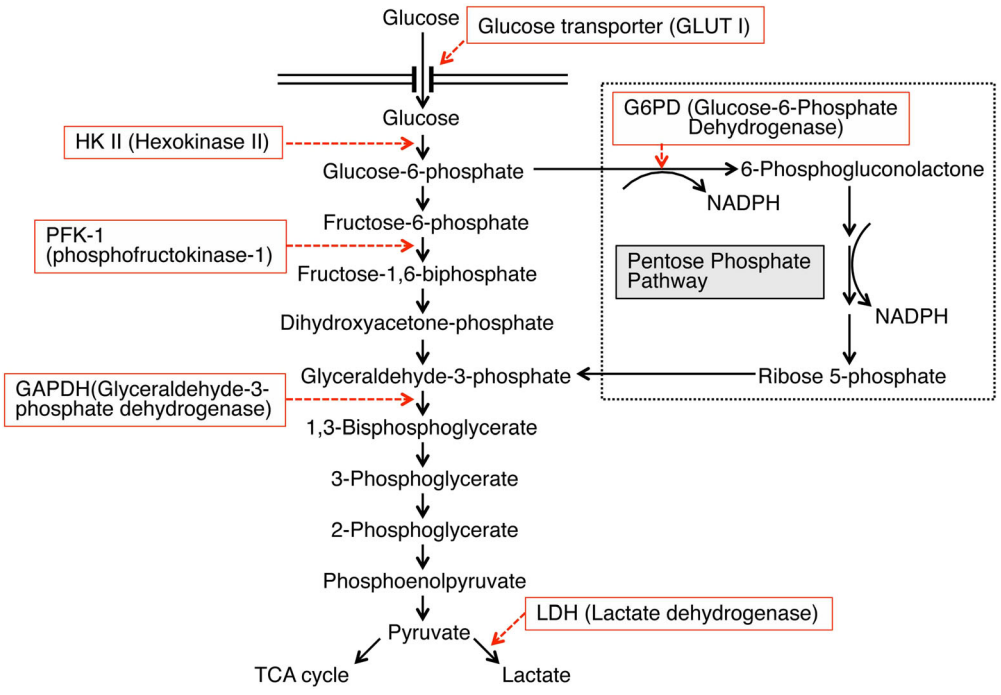

FIGURE 1 Glycolysis and glycolytic enzymes

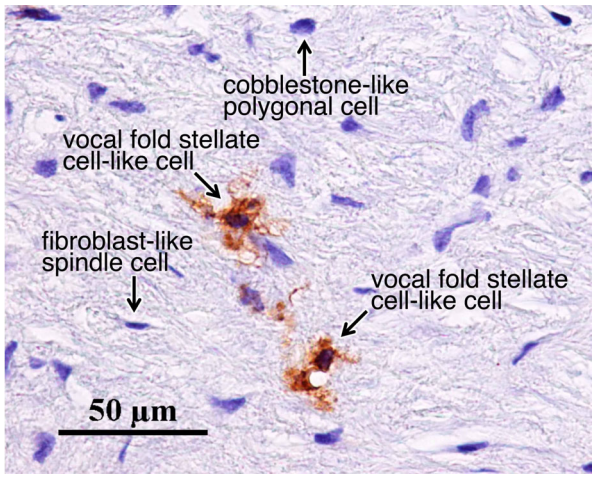

FIGURE 2 Glucose transporter-1 (GLUT-1) immunohistochemical staining of the cells in the maculae flavae of the human adult vocal fold. Vocal fold stellate cell-like cells strongly expressed GLUT-1

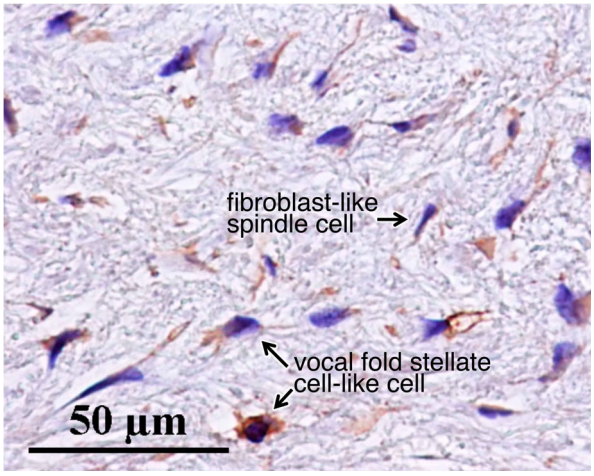

FIGURE 3 Hexokinase II (HK II) immunohistochemical staining of the cells in the maculae flavae of the human adult vocal fold. All phenotypes of cells expressed HK II and some vocal fold stellate cell-like cells strongly expressed HK II

antibody against LDHA (Abcam, Cambridge, UK, ab101562, rabbit monoclonal) were used.

Specimens were sectioned to a thickness of 5 to 6 µm and mounted on glass slides. Deparaffinized and hydrated sections were rinsed with 0.01-mol/L phosphate buffered saline (PBS) at pH 7.4. The specimens were covered with 3% hydrogen peroxide for 10 minutes and rinsed with 0.01-mol/L PBS, followed by treatment with normal mouse serum. The specimens were then incubated with the primary antibody for 60 minutes at 4°C.

After rinsing with PBS and labeling with the universal immuno-enzyme polymer method staining kit, a color reaction was developed with 3,3′-diaminobenzidine at room temperature. Immunoreactivity was examined by light microscopy.

3 | RESULTS

3.1 | Glucose transporter of the cells in the maculae flavae of the human vocal fold mucosa

Among the phenotypes of cells in the human adult maculae flavae, the vocal fold stellate cell-like cells strongly expressed GLUT1 (Figure 2). Other phenotypes of cells in the human maculae flavae sparsely expressed GLUT1.

3.2 | Hexokinase (catalyzes glucose into glucose-6-phosphate) in the cells of the maculae flavae of the human vocal fold mucosa

All phenotypes of cells in the human maculae flavae expressed HK II and some vocal fold stellate cell-like cells strongly expressed HK II (Figure 3).

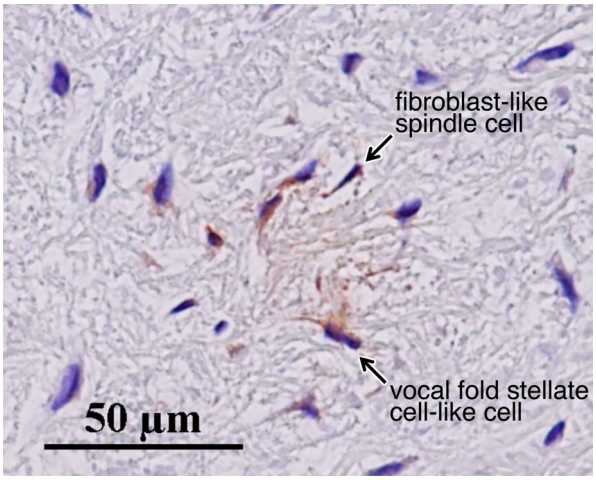

FIGURE 4 Glucose-6-phosphate dehydrogenase (G6PD) immunohistochemical staining of the cells in the maculae flavae of the human adult vocal fold

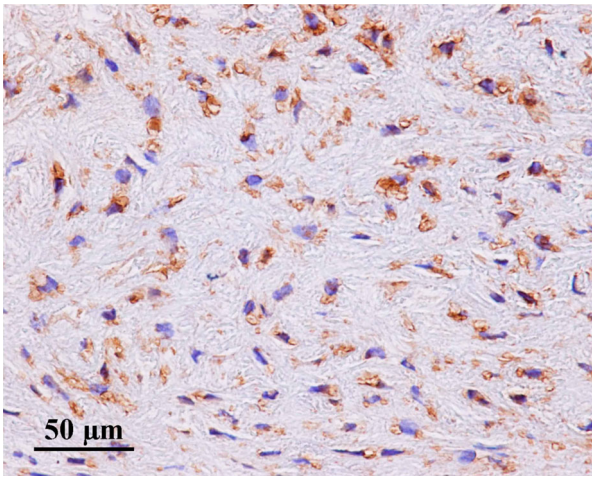

FIGURE 5 Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) immunohistochemical staining of the cells in the maculae flavae of the human adult vocal fold

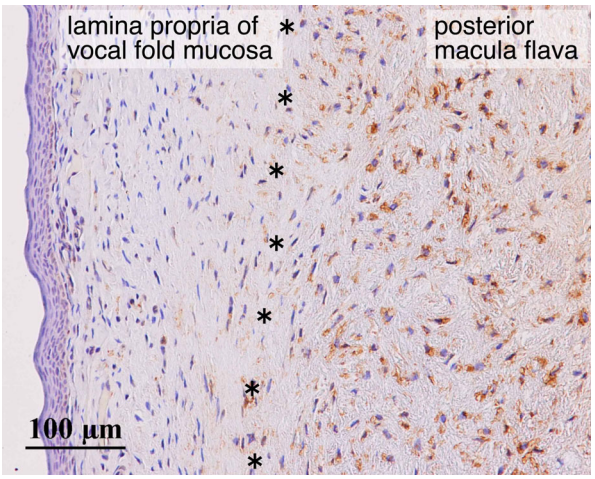

FIGURE 6 Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) immunohistochemical staining of the cells in the maculae flavae and lamina propria of the human adult vocal fold mucosa. Cells in the human maculae flavae strongly expressed GAPDH. On the other hand, stratified squamous epithelium and interstitial cells in the lamina propria of the vocal fold mucosa sparsely expressed GAPDH. (*: Border between posterior macula flava and lamina propria of the vocal fold mucosa)

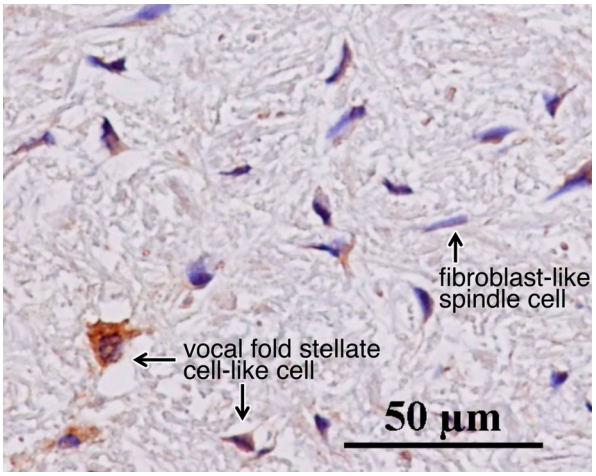

FIGURE 7 Lactate dehydrogenase A (LDHA) immunohistochemical staining of the cells in the maculae flavae and lamina propria of the human adult vocal fold mucosa. All phenotypes of cells expressed LDHA and some vocal fold stellate cell-like cells strongly expressed LDHA

3.3 | Phosphofructokinase-1 (catalyzes fructose-6-phosphate into fructose-1,6-biphosphate) in the cells of the maculae flavae of the human vocal fold mucosa

The cells in the human maculae flavae did not express phosphofructokinase-1.

3.4 | Glucose-6-phosphate dehydrogenase (catalyzes Glucose-6-phosphate into 6-phosphogluconolactone) in the cells of the maculae flavae of the human vocal fold mucosa

The cells in the human maculae flavae expressed G6PD (Figure 4), rate-limiting enzyme of the pentose phosphate pathway.

3.5 | Glyceraldehyde-3-phosphate dehydrogenase (catalyzes glyceraldehyde-3-phospate into 1,3-bisphosphoglycerate) in the cells of the maculae flavae of the human vocal fold mucosa

All phenotypes of cells in the human maculae flavae strongly expressed GAPDH (Figure 5). On the other hand, interstitial cells in the lamina propria of the vocal fold mucosa sparsely expressed GAPDH (Figure 6).

3.6 | Lactate dehydrogenase A (catalyzes pyruvate into lactate) in the cells of the maculae flavae of the human vocal fold mucosa

All phenotypes of cells in the human maculae flavae expressed LDHA and some vocal fold stellate cell-like cells strongly expressed LDHA (Figure 7). Consequently, the maculae flavae of the human adult vocal fold was likely to be an anaerobic microenvironment.

4 | DISCUSSION

Our previous studies showed that the tissue stem cells in the maculae flavae (stem cell niche) of the human vocal fold have cellular heterogeneity and hierarchy in the stem cell system in vivo.[11,12] Regarding heterogeneity, cobblestone-like polygonal cells, vocal fold stellate cell-like cells possessing lipid droplets in the cytoplasm and fibroblast-like spindle cells were intermingled in the maculae flavae, indicating that three phenotypes of cells are present in the human adult maculae flavae in vivo.[11,12] As for hierarchy, cobblestone-like polygonal cells are likely at the top and fibroblast-like spindle cells are likely at the bottom of the cellular hierarchy in the stem cell system.[11,12] Therefore, vocal fold stellate cell-like cells are likely at the second level of the cellular hierarchy.[11,12] This suggests that the vocal fold stellate cell-like cells are likely progenitor cells or transient amplifying cells in the stem cell system of the human vocal fold mucosa.[11,12]

Our previous research using transmission electron microscopy suggested that the tissue stem cells in the human maculae flavae seem to rely more on glycolysis for energy supply in comparison with oxidative phosphorylation from the aspect of mitochondrial microstructure.[9]

The present study is also consistent with the hypothesis that the tissue stem cells in the human maculae flavae rely more on anaerobic glycolysis for energy supply in comparison with oxidative phosphorylation. Furthermore, among the three phenotypes of cells in the human adult maculae flavae in vivo, anaerobic glycolytic activity of some vocal fold stellate cell-like cells were likely to be high.

4.1 | Glucose transporter of the tissue stem cells in the maculae flavae of the human vocal fold mucosa

Due to its polar nature and large size, glucose molecules cannot transverse the lipid membrane of cells by simple diffusion.[18] Instead, the entry of glucose molecules into the cells is effected by a large family of structurally related transport proteins known as glucose transporters (GLUTs).[18] The GLUTs transport glucose across the plasma membrane by means of a facilitated diffusion mechanism.[18] The function of glucose sensing has two components: (a) entry of glucose into the cell mediated by GLUTs and (b) metabolism of glucose through phosphorylation by glucokinase.[18] Availability of glucose for glycolysis is controlled by transport into the cells, which intern is regulated by insulin.[19]

The present study showed that, among the three phenotypes of cells in the human maculae flavae, the vocal fold stellate cell-like cells strongly expressed GLUT1. Since the glucose uptakes are regulated by GLUT, the vocal fold stellate cell-like cells likely have the highest glycolytic activity among the three phenotypes of cells in the human maculae flavae.

After glucose enters into the cells, glucose is catalyzed by cytosolic glycolytic enzymes.[19] The first step in glucose metabolism pathways is that glucose enters glycolysis by phosphorylation to glucose-6-phosphate, catalyzed by hexokinase, using ATP as the phosphate donor.[19] Hexokinase has a high affinity for glucose.[19]

The present study showed that all three phenotypes of cells in the human maculae flavae expressed hexokinase II and some vocal fold stellate cell-like cells strongly expressed hexokinase II.

4.2 | Pentose phosphate pathway of the glycolysis of the tissue stem cells in the maculae flavae of the human vocal fold mucosa

The pentose phosphate pathway is an alternative route for the metabolism of glucose.[20] It does not lead to formation of adenosine triphosphate (ATP) but has two major functions: (a) the formation of NADPH for synthesis of fatty acid and steroids and maintaining reduced glutathione for antioxidant activity, and (b) the synthesis of ribose for nucleotide and nucleic acid formation.[20] Therefore, NADPH works as a reducing agent.

The present study showed that the cells in the human maculae flavae expressed G6PD, the rate-limiting enzyme of the pentose phosphate pathway. The tissue stem cells in the human maculae flavae seem to rely more on glycolysis, especially by the pentose phosphate pathway, for energy supply.

4.3 | Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the glycolysis of the tissue stem cells in the maculae flavae of the human vocal fold mucosa

Glyceraldehyde-3-phosphate is reduced to 1,3-bisphosphoglycerate catalyzed by GAPDH.[9] GAPDH has been recognized as an important enzyme for energy metabolism and the production of ATP through anaerobic glycolysis in the cytoplasm.[21] Recent studies have shown that GAPDH has multiple functions independent of its role in energy metabolism.[21] Although increased GAPDH gene expression and enzymatic function is associated with cell proliferation and tumorigenesis, conditions such as oxidative stress impair GAPDH catalytic activity and lead to cellular aging and apoptosis.[21] The resultant blockade of glycolysis through peroxide-induced inhibition of GAPDH facilitates the flow of glucose equivalents through the pentose phosphate pathway, thus increasing the availability of NADPH during oxidative stress.[22]

The present study showed that the cells in the human maculae flavae expressed GAPDH. GAPDH is a glycolytic enzyme for energy metabolism and the production of ATP through anaerobic glycolysis in the cells in the human maculae flavae. However, other functions of GAPDH independent of its role in energy metabolism of the vocal fold remain ambiguous.

4.4 | Reduction of pyruvate to lactate in the glycolysis of the tissue stem cells in the maculae flavae of the human vocal fold mucosa

The availability of oxygen determines which of the two pathways is followed (Figure 1).[19] Under anaerobic conditions, NADH cannot be reoxidized thorough the respiratory chain, and pyruvate is reduced to lactate catalyzed by lactate dehydrogenase.[19] Under aerobic conditions, pyruvate is transported into the mitochondria and undergoes oxidative decarboxylation to acetyl-CoA.[20]

The present study showed that all three phenotypes of cells in the human maculae flavae expressed lactate dehydrogenase and some vocal fold stellate cell-like cells strongly expressed lactate dehydrogenase. Consequently, pyruvate is likely to be reduced to lactate catalyzed by lactate dehydrogenase under anaerobic microenvironment in the tissue stem cells in the maculae flavae of the human vocal fold.

4.5 | Glycolysis of the tissue stem cells in the human maculae flavae

Oxidative stress shortens the life span of stem and progenitor cells, among which reactive oxygen stress (ROS) is the most common threat

and ROS accelerates aging through random and sequential damage to cell components.[23]

ROS is continuously generated by normal metabolic processes such as oxidative phosphorylation.[24] Oxidative phosphorylation in the mitochondria is the major source of endogenous ROS.[24] 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.[24]

Quiescence (G_0 phase) is critical for protecting the stem cell compartment.[23] The quiescent state is generally viewed as a mechanism for avoiding accumulation of damage resulting from physiological stress including oxidative stress.[25] Our previous investigations showed that most of the cells in the maculae flavae of the vocal fold do not express Ki-67, indicating that they are resting cells (G_0 phase).[1] Consequently, this suggests the cells in the maculae flavae avoid accumulation of damage resulting from oxidative stress.

In this study, the tissue stem cells in the human maculae flavae seem to rely more on anaerobic glycolysis using the pentose phosphate pathway for energy supply in comparison with oxidative phosphorylation indicating the intracellular ROS production is suppressed. The metabolism of the tissue stem cells in the human maculae flavae is likely to be favorable to maintaining the stemness and undifferentiated states of the cells in the stem cell system.

5 | CONCLUSIONS

The present study is consistent with the hypothesis that the tissue stem cells in the maculae flavae of the human vocal fold rely more on anaerobic glycolysis, especially by the pentose phosphate pathway, for energy supply. The metabolism of the tissue stem cells in the human maculae flavae is likely to be favorable to maintaining the stemness and undifferentiated states of the tissue stem cells in the stem cell system.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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