ABSTRACT

Objectives: There is growing evidence that vocal fold stellate cells in the maculae flavae (MFe) are tissue stem cells or progenitor cells of the human vocal fold, and that the MFe are a stem cell niche. The origin of the cells in the human MFe and the relationship with bone marrow derived cells was investigated.

Methods: Five adult vocal fold mucosae were investigated. Immunoreactivity to antibodies directed to cytokeratin, desmin, GFAP, vimentin, CD34, CD45 and collagen type I was investigated. The cells in the human MFe were subcultured and morphological features were assessed.

Results: Cultured cells in the human MFe formed a colony-forming unit, indicating they are mesenchymal stem cells or stromal stem cells in the bone marrow. The cells in the human MFe expressed hematopoietic markers (CD34, CD45) and collagen type I, which are the major markers for bone marrow derived circulating fibrocyte. The cultured cells in the human MFe expressed epithelium-associated (cytokeratin), muscle-associated (desmin), neural-associated (GFAP), and mesenchymal cell-associated (vimentin) proteins, indicating the cells in the human MFe are undifferentiated and express proteins of all three germ layers.

Conclusions: The cells in the human MFe arise from the differentiation of bone marrow cells. The cells in the human MFe are undifferentiated cells derived from the bone marrow. The results of this study are consistent with the hypothesis that the cells, including VFSCs, are tissue stem cells or progenitor cells of the human vocal fold.

Key Words: vocal fold stellate cell, tissue stem cell, progenitor cell, human vocal fold, larynx

INTRODUCTION

Human maculae flavae located at both ends of the vocal fold mucosa are involved in the metabolism of extracellular matrices, which are essential for the viscoelastic properties of the lamina propria of the human vocal fold. Human adult maculae flavae are responsible for maintaining the characteristic layered structure of the human vocal fold mucosa. Human newborn, infant and child maculae flavae are responsible for forming the characteristic layered structure of the human vocal fold mucosa. Human maculae flavae are an important structure in the growth, development and aging of the human vocal fold mucosa.

Many Vocal Fold Stellate Cells (VFSCs) (Sato et al, 2001), which store vitamin A in their lipid droplets, are the major makers for bone marrow derived circulating fibrocyte. The VFSCs in the maculae flavae are considered a new category of cells in the human vocal fold. Adult tissue-specific stem cells (tissue stem cells) have the capacity to self-renew and to generate functionally differentiated cells that replenish lost cells throughout an organism’s lifetime. There is growing evidence that the VFSCs in the human maculae flavae are tissue stem cells or progenitor cells in the human vocal fold mucosa. The human maculae flavae are a candidate for a stem cell niche, which is a microenvironment nurturing a pool of stem cells which, in this case, are VFSCs.

As a result of this heterogeneity, it is uncertain whether the VFSCs derive from the same embryonic source as conventional fibroblasts in the human vocal fold mucosa. In this study, the origin of the VFSCs in the human maculae flavae, especially regarding the relationship with bone marrow derived cells, was investigated.

METHODS AND MATERIALS

Five normal human adult larynges from surgical specimens and autopsy cases were used.

1) Human vocal fold stellate cell culture

After extraction of the anterior macula flava of the human vocal fold mucosa from surgical specimens under microscope, it was minced and cultured in an MF-start primary culture medium (Toyobo, Osaka, Japan). After the primary culture, MF-medium (Mesenchymal Stem Cell Growth Medium) (Toyobo, Osaka, Japan) was used to proliferate the cells.

2) Immunohistochemical investigations of the cultured human vocal fold stellate cells

For light microscopy, cultured VFSCs were fixed in 4% paraformaldehyde on the glass slides for cell culture and immunohistochemical staining was carried out. Cytokeratin, vimentin, glial fibrillary acidic protein (GFAP) and desmin were detected histochemically by immunohistochemistry, for which a universal immuno-enzyme polymer method staining kit (Histofine Simple Stain MAX PO, Nichirei, Tokyo, Japan) was used. These phenomena suggest that cell division in the human maculae flavae is an asymmetric self-renewal. Asymmetry in cell division gives rise to the possibility that the human maculae flavae contain tissue stem cells (Figure 3). The VFSCs are possibly transit-amplifying cells (progenitor cell) (Figure 3).

RESULTS

1) Human vocal fold stellate cell culture

After 3 days of primary culture in an MF-start primary culture medium, two types of cells, which were fibroblast-like cells and cobblestone-like squamous cells, grew from the maculae flava fragments (Figure 1). After removing the two types cells by cell scraper, each cell was cultured in an MF-medium (Mesenchymal Stem Cell Growth Medium) to proliferate the cells.

After 6 days of first subculture, subcultured fibroblast-like cells became stellate in shape and possessed slender cytoplasmic processes (Figure 2). Small lipid droplets were present in the cytoplasm (Figure 2). After 6 days of second subculture, subcultured cobblestone-like squamous cells formed a colony-forming unit (Figure 2), indicating these cells are mesenchymal stem cells or stromal stem cells in the bone marrow.

2) Immunohistochemical investigations of the cultured human vocal fold stellate cells

Cytoplasmic cytokeratin (epithelium-associated protein), vimentin (mesenchymal cell-associated protein), GFAP (neural-associated protein) and desmin (muscle-associated protein) immunoreactivity were present in the cells, which became VFSCs (Figure 4A) and cells which formed a colony-forming unit (Figure 4B), indicating these cells were undifferentiated and expressed proteins of all three germ layers.

CONCLUSIONS

The cells, including the VFSCs, in the human maculae flavae arise not from resident interstitial cells but from the differentiation of bone marrow cells. The cells, including the VFSCs, in the human maculae flavae are undifferentiated cells derived from the bone marrow.

The results of this study are consistent with the hypothesis that the cells, including the VFSCs, are tissue stem cells or progenitor cells of the human vocal fold.

REFERENCES