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Expression of podoplanin in the mouse salivary glands

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ABSTRACT

Objective: Podoplanin is one of the most highly expressed lymphatic-specific genes. Here, we report the distribution of cells expressing podoplanin in mouse salivary glands.
Design: We immunohistochemically investigated the distribution of cells expressing podoplanin in mouse major salivary glands by laser-scanning microscopy. The expression of endothelial cell marker PECAM-1 was tested to discriminate lymphatic endothelium from salivary gland cells, and myoepithelial cells were identified by an antibody for P-cadherin.
Results: The podoplanin expression was rarely found in acini of the parotid gland but clearly found at the basal portion of acini in the submandibular and sublingual glands. The number of portion reacted with anti-podoplanin is greater in the sublingual gland than in the submandibular gland. The expression was also found at the basal portion of ducts in all major salivary glands. The P-cadherin expression was rarely found in acini of the parotid gland but found in acini of the sublingual gland and on ducts in parotid and sublingual glands, corresponding to the area of podoplanin expression.
Conclusions: It was suggested that the acinar and myoepithelial cells in the salivary glands have the ability to express podoplanin, and that the expression may be concerned with the mucous saliva excretion.

1. Introduction

Podoplanin, a 43-kDa transmembrane glycoprotein, is one of the most highly expressed lymphatic-specific genes and is regulated by homeobox gene *Prox1*.[1–4] The podoplanin expression in lymphatic endothelium was first reported as E11 antigen and was further identified as podoplanin because of the expression in kidney glomerular epithelial cells (podocytes).[5,6] *Podoplanin* is homologous to *T1α* encodes an antigen expressed at alveolar type I cells in rat lung.[7,8] *Podoplanin−/−* mice die at birth because of respiratory defect and congenital lymphedema due to the failure in lymphatic pattern formation. In the level of cultured cell it has been indicated that the lymphatic tube formation is based on the cell adhesion with podoplanin at the cell–cell contact.[9]

On the other hand it is well established that the mouse parotid, submandibular, and sublingual glands are composed of serous, seromucous, and mucous-dominant mixed acinar cells, respectively.[10–12] The determinant for the viscosity of saliva is the glycoprotein mucins which protect mucosal cell membranes against proteases. The submandibular gland secretes 30% of the salivary mucins while sublingual and a large number of minor glands of palate, cheeks and lips secrete 70%. Concentration of mucin secreted by the sublingual gland is higher than that secreted by the submandibular gland, while the secretion of parotid gland is almost devoid of mucins.[13–15] It has been reported that podoplanin is resistant to proteases because of the negatively charged mucin-type protein.[6,14] It is thought that the expression of transmembrane protein podoplanin may contribute to pro-

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tecting salivary gland cells from protease by covering the cell surface with mucin. The expression of podoplanin in the several epithelial cells, such as epidermis and alveolar epithelia, has been noticed but little is known on the expression in salivary glands.[16,17]

Platelet-endothelial cell adhesion molecule-1 (PECAM-1) is a well-established endothelial cell marker generally expressed on leukocytes and endothelial cells. The PECAM-1 is a 130-kDa type I transmembrane glycoprotein belonging to the immunoglobulin superfamily and acts as an adhesion molecule.[18–21] In this study the expression of PECAM-1 was immunohistochemically tested to discriminate lymphatic vessels from salivary gland cells since the lymphatic endothelium expresses both podoplanin and PECAM-1. The study here was designed to investigate the distribution of cells expressing podoplanin in mouse major salivary glands.

2. Materials and methods

Eight-week-old wild-type male mice (C57BL6/J, $n = 5$) purchased from the Jackson Laboratory (Bar Harbor, ME, USA) were used. The collection of the tissue was conducted after euthanasia by intraperitoneal injection with sodium pentobarbital (10 ml/kg, Nembutal, Abbott Laboratories, North Chicago, IL). Mice were perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The protocol for animal use was reviewed and approved by the animal experiment committee of Fukuoka Dental College, Fukuoka, Japan. The frozen 10 μm sections were cut in a cryostat and fixed in 5% formalin–PBS containing 0.1% glutaraldehyde for 10 min at 4 °C. After the treatment with 0.1% rabbit serum the sections were treated with a cocktail of antibodies: 0.5 μg/ml of goat anti-mouse podoplanin (AngioBio Co., Del Mar, CA) and rat anti-mouse PECAM-1 (SouthernBiotechnology Associates, Inc., Birmingham, AL), or goat anti-mouse podoplanin (AngioBio) and rat anti-mouse P-cadherin (R&D Systems Inc., Minneapolis, MN) for 8 h at 4 °C. After reacting with first antibodies, the sections were immunostained for 0.5 h at 20 °C with a cocktail of second antibodies (0.1 μg/ml): Alexa Fluor (AF) 488 or 568-conjugated rabbit anti-goat or anti-rat IgGs (Probes Invitrogen Com., Eugene, OR), and examined by laser-scanning microscopy (Axiovert 135M, Carl Zeiss, Jena, Germany) with a ×52 oil planapochromatic objective lens (numerical aperture ×1.3).

3. Results

3.1. *Immunohistochemistry of the mouse kidney and tongue*

The mouse kidney and tongue tissue was used to test the specificity of anti-podoplanin (Fig. 1). The immunostaining with anti-PECAM-1 was simultaneously performed to identify lymphatic vessels because anti-podoplanin also reacts to the lymphatic endothelium. Reaction products with anti-PECAM-1 were only detected on blood vessels including glomerular arteriole and on lymphatic vessels. Reaction products with anti-podoplanin were only detected on podocytes and lymphatic vessels.

3.2. *Immunohistochemistry of the mouse major salivary glands*

In the mouse parotid gland the terminal secretory end pieces of acini were densely composed of serous pyramidal cells with basophilic cytoplasm (Fig. 2). The acini had the intercalated

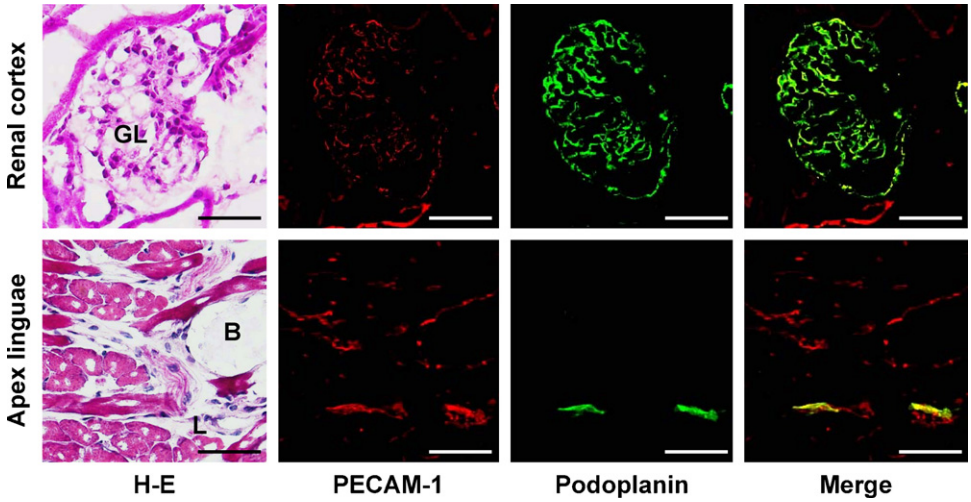

Fig. 1 – Expression of podoplanin in the mouse kidney and tongue. Reaction products with anti-PECAM-1, and anti-podoplanin are visualized with AF568-conjugated (in red), and AF488-conjugated (in green) second antibodies. In the glomerulus (GL) of renal cortex the merged image indicates that reaction products with anti-PECAM-1 and anti-podoplanin are found on glomerular arteriole but absent on uriniferous tubules. In the apex linguae the merged image indicates that strong reaction products with anti-PECAM-1 are found on blood (B) and lymphatic vessels (L), but not in the muscle and connective tissue. The merged image also indicates that the reaction products with anti-podoplanin and anti-PECAM-1 are found on lymphatic vessels only (in yellow). Bar, 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)