Impaired Junctions and Invaded Macrophages in Oral Epithelia With Oral Pain

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Summary

Recurrent or chronic oral pain is a great burden for patients. Recently, the links between epithelial barrier loss and disease were extended to include initiation and propagation. To explore the effects of pathohistological changes in oral epithelia on pain, we utilized labial mucosa samples in diagnostic labial gland biopsies from patients with suspected Sjögren's syndrome (SS), because they frequently experience pain and discomfort. In most labial mucosa samples from patients diagnosed with SS, disseminated epithelial cellular edema was prevalent as ballooning degeneration. The disrupted epithelia contained larger numbers of infiltrating macrophages in patients with oral pain than in patients without pain. Immunohistochemistry revealed that edematous areas were distinct from normal areas, with disarranged cell–cell adhesion molecules (filamentous actin, E-cadherin, β -catenin). Furthermore, edematous areas were devoid of immunostaining for transient receptor potential channel vanilloid 4 (TRPV4), a key molecule in adherens junctions. In an investigation on whether impaired TRPV4 affect cell–cell adhesion, calcium stimulation induced intimate cell–cell contacts among oral epithelial cells from wild-type mice, while intercellular spaces were apparent in cells from TRPV4-knockout mice. The present findings highlight the relationship between macrophages and epithelia in oral pain processing, and identify TRPV4-mediated cell–cell contacts as a possible target for pain treatment. (J Histochem Cytochem 67:245–256, 2019)

Keywords

human, mucosal immunity, oral manifestations, transient receptor potential channels

Introduction

The health and psychosocial burdens associated with chronic oral pain and discomfort are great challenges in dental practice. Chronic mucosal hypersensitivity is considered to be associated with hyposalivation,¹ damaged neurons,^{2,3} and increased supply of pain nerves.⁴ However, the underlying mechanisms remain to be elucidated.

Current evidence suggests that impairment of the barrier system is related to persistent pain or chronic

irritable sensation of the skin and colon.^{5–7} The outermost barriers of the body are covered with epithelia, and epithelial continuity is largely dependent on proper cell–cell adhesion complexes that provide an internal

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homeostatic environment with appropriate cellular proliferation and migration. Non-epithelial immune cells such as Langerhans cells, T lymphocytes, and resident or non-resident macrophages are also present in the mucosa and skin.⁸

We hypothesized that chronic oral pain or discomfort may be related to impairment of the epithelial barrier. To test this hypothesis, we utilized labial mucosa samples from patients with Sjögren's syndrome (SS), because SS patients commonly manifest persistent pain or discomfort in the oral cavity.⁹ Although SS is a common systemic autoimmune disease characterized by lymphocytic infiltration in the salivary and lacrimal glands,¹⁰ few reports have focused on the mucosal membrane.

Transient receptor potential vanilloid 4 (TRPV4), a member of the TRP ion channel superfamily, permeates cations in response to diverse stimuli such as hypo-osmolality, mechanical stress, chemicals, and temperature changes.¹¹ We previously demonstrated that oral epithelia express TRPV4 and become activated by warm temperatures via TRPV4.^{12,13} TRPV4 was also reported to be a key molecule in the regulation of intercellular junctions.¹⁴ Moreover, TRPV4 is a pharmacological target for irritabilities associated with barrier disruption in the skin and colon.¹⁵

In the present study, we explored the relationship between oral pain and the oral mucosal epithelial barrier by observing the histology of labial mucosa samples. We found that inflammatory cell infiltrations were associated with impaired cell–cell contacts in the epithelia, and identified a possible relationship between pain manifestation and TRPV4 expression.

Materials and Methods

Patients

The experimental protocol was approved by the local institutional review board and independent ethical committee. All patients provided written informed consent. Labial mucosa samples were obtained from 25 patients with clinically suspected SS (one male, 24 females; age range: 23–78 years) during labial gland biopsy procedures¹⁶ at Kyushu University Hospital and Saga University Hospital from 2013 to 2016. We also utilized resected tissues from seven patients with mucous cysts (two males, five females; age range: 18–44 years), with the expectation of normal histology in the epithelial layer. The suspected SS patients underwent a clinical test for their salivary flow rate and an autoantibody anti-SS-A/Ro examination before the biopsy. Medical records were reviewed to collect clinical data, including presence of pain in the oral cavity.

The Saxon test and the spitting method were performed to evaluate the stimulated and unstimulated salivary flow rate, respectively. Patients were diagnosed according to the criteria proposed by the Research Committee on SS of the Ministry of Health and Welfare of the Japanese Government and the criteria proposed by the National Institutes of Health (NIH)-funded Sjögren's International Collaborative Clinical Alliance.^{17,18} Of the 25 suspected SS patients, 19 were diagnosed as definitive SS and six patients were negative. The clinicopathological characteristics of the patients are summarized in Table 1.

Immunohistochemistry

Labial mucosae from the wet area of the lower lip were fixed with 4% paraformaldehyde in phosphate buffer (pH 7.4) immediately after the biopsy procedure¹⁶ or cyst resection. The tissues were processed for hematoxylin-eosin staining and immunohistochemistry as described previously.¹⁹ Primary antibodies against TRPV4 (1:500; ab74738; Abcam, Cambridge, UK), E-cadherin (1:200; M108; Takara Bio Inc., Shiga, Japan), β -catenin (1:200; #9592; Cell Signaling Technology, Beverly, MA), ionized calcium-binding adapter molecule 1 (Iba1; 1:500; 019-19741; Wako Pure Chemical Industries, Ltd., Osaka, Japan), CD11b (1:200; MCA275R; AbD Serotec, Oxford, UK), CD207 (1:200; ab49730; Abcam), and PGP9.5 (1:1000; RA-95101; Ultracclone, Isle of Wight, UK) were used. The specificity of the anti-TRPV4 antibody was validated using C57BL/6 wild-type (WT) mice and TRPV4-deficient (TRPV4KO) mice.²⁰ Immunoreactivity for TRPV4 was clearly observed in the palatal epithelium of WT mice, but was faint in the palatal epithelium of TRPV4KO mice (data not shown). After incubation with the primary antibodies, the samples were washed and incubated with Alexa 488-conjugated donkey anti-rabbit IgG, Alexa 594-conjugated donkey anti-rat IgG (1:200; Jackson ImmunoResearch, West Grove, PA), or Alexa 568-conjugated goat anti-mouse IgG (1:200; Molecular Probes, Eugene, OR) secondary antibodies. Actin fibers were labeled with Phalloidin-Alexa 568 (1:100; Invitrogen, Carlsbad, CA) and the nucleus was stained with DAPI (Dojindo Laboratories, Kumamoto, Japan). Images were acquired using a light microscope (BX51; Olympus, Tokyo, Japan) and a confocal laser scanning microscope (LSM510; Carl Zeiss, Oberkochen, Germany). All images were analyzed with ImageJ software (NIH, Bethesda, MD). The numbers of intraepithelial Iba1-positive cells were counted in three randomly selected areas in each sample (labial gland biopsy: $n=25$; resected mucous cyst: $n=7$) and averaged.

Table 1. Clinicopathological Characteristics and Histological Findings of 25 Patients Evaluated in This Study.

No.	Age	Sex	Diagnosis	Oral Pain	Oral Dryness	Stimulated Saliva	Unstimulated	Anti-SS-A/Ro	Epithelial Cellular Edema	Intraepithelial Iba I+ Cell (/mm ²)
						Flow (ml/2 min)	Saliva Flow (ml/15 min)			
1	63	F	SS definitive	+	+	1.16	0.15	+	severe	162
2	53	F		+	+	5.20	2.20	+	mild	300
3	71	F		+	+	0.88	0.36	+	–	320
4	73	F		+	+	0.15	0.10	–	mild	351
5	62	F		+	+	1.52	0.25	–	severe	369
6	57	F		+	+	2.98	1.40	+	severe	425
7	49	F		+	+	0.43	0.50	+	mild	466
8	54	F		+	+	0.54	0.00	+	severe	669
9	58	F		–	+	0.40	0.85	+	–	122
10	45	F		–	+	0.66	1.20	+	severe	140
11	45	F		–	+	0.95	0.10	+	severe	161
12	78	F		–	–	1.22	1.80	–	mild	202
13	49	F		–	–	5.43	5.00	+	severe	244
14	69	M	SS negative	–	+	3.70	6.92	+	severe	247
15	34	F		–	–	3.49	4.40	–	mild	247
16	41	F		–	+	1.59	0.23	+	mild	275
17	65	F		–	–	3.19	1.20	+	severe	326
18	40	F		–	–	2.57	1.40	+	mild	363
19	74	F		–	+	1.38	0.50	+	mild	435
20	68	F		+	+	4.87	0.20	+	severe	165
21	69	F		+	+	3.19	3.00	–	–	349
22	48	F		–	+	0.66	0.60	–	–	207
23	68	F		–	+	1.79	1.10	+	mild	211
24	23	F		–	–	0.74	1.20	–	severe	320
25	58	F		–	–	3.05	5.75	+	mild	366
26	19	F	Mucous cyst	NA	NA	NA	NA	NA	–	62
27	20	F							–	77
28	44	M							–	119
29	23	F							–	127
30	18	F							–	150
31	37	F							–	262
32	26	M							–	329

Abbreviations: SS, Sjögren's syndrome; Iba I, ionized calcium-binding adapter molecule I.

Cell Culture and Ca²⁺ Switch Experiment

The animal protocol was approved by the Animal Care and Use Committee of Kyushu University. WT mice and TRPV4KO mice²⁰ were housed with free access to food and water in a specific pathogen-free and temperature-controlled room on a 12-hr/12-hr light/dark cycle. Oral epithelial cells were isolated as described.¹² Briefly, palates were dissected from mice on postnatal days 2 to 5 ($n=8$ per group) and incubated in 400 U/ml dispase I (Godoshusei Co. Ltd., Tokyo, Japan) at 4°C overnight. The epithelia were peeled off and treated with 0.25% trypsin (Invitrogen). After gentle pipetting and centrifugation, the cell pellet was collected and resuspended in

CnT-Prime medium (CELLnTEC, Bern, Switzerland). Cells (0.8×10^4) were seeded on coverslips. To investigate the formation of cell–cell adhesions, cells cultured for 3 days in medium containing 0.07 mM CaCl₂ were stimulated with medium containing 1.2 mM CaCl₂ for 24 hr and subjected to immunocytochemistry. Mouse gingival epithelial cell line GE1²¹ cells were cultured in SFM101 medium (Nissui, Tokyo, Japan) supplemented with 1% fetal bovine serum (Invitrogen) and 10 ng/ml mouse epidermal growth factor (Sigma-Aldrich, St. Louis, MO) with or without stimulation with a TRPV4 agonist (GSK1016790A; Sigma-Aldrich) for 10 min or a TRPV4 antagonist (GSK2193874; Sigma-Aldrich) for 24 hr.

Data Analysis

Data were expressed as mean $\pm$ standard error. The statistical significance of differences in data were analyzed by a *t*-test using Excel software (Microsoft Inc., Redmond, WA). Values of $p < 0.05$ were considered significant.

Results

Macrophages Infiltrate the Degenerated Mucosa and Cause Oral Pain

To estimate the immune cell distribution in the labial mucosa, biopsy specimens were immunolabeled with antibodies against Iba1, CD11b, and CD207/Langerin as markers of pan-macrophages,²² monocyte/immature macrophages,²³ and Langerhans cells,²⁴ respectively. We found that a substantial number of Iba1-immunoreactive cells were distributed over the labial mucosa from SS patients in not only connective tissues but also epithelia (Fig. 1A). We also observed abundant Iba1-positive cells infiltrating the glandular lesions in SS patients (data not shown). Meanwhile, in the mucosa of mucous cysts, there were few intraepithelial Iba1-positive cells, despite the abundant number of inflammatory cells in the lamina propria (Fig. 1A). The number of intraepithelial Iba1-positive cells was significantly larger in SS patients than in mucous cyst patients (Fig. 1B). Because the Iba1-positive cells showed a dendritic morphology, we double-labeled these cells with the Langerhans cell marker CD207. A few Iba1-positive cells were CD207-positive, but the majority were CD207-negative (Fig. 1C). Infiltration of CD11b-positive cells, which were circular in shape, was also observed, with cell aggregates in the lamina propria and some cells in the epithelia. Microglia or macrophages are known to change their shape from ramified to amoeboid upon activation.²⁵ Interestingly, amoeboid-shaped Iba1-positive cells were adjacent to CD11b-positive cells, suggesting direct contact between these cells (Fig. 1C).

Next, we examined the relationships between clinical signs and histological characteristics. Larger numbers of Iba1-positive cells were found in patients with oral pain (Fig. 1D). Unexpectedly, the salivary flow rates and subjective feelings of oral dryness did not show significant correlations with the number of intraepithelial Iba1-positive cells (Fig. 1D). Immunostaining for the pan-neuronal marker PGP9.5 was performed to examine the relationship between nerve distribution and pain. A PGP9.5-immunoreactive nerve plexus was found in the lamina propria and a few nerve fibers penetrated into the epithelia. We did not find any clear differences in the nerve distributions

between SS patients and mucous cyst patients (Supplemental Fig. 1A).

Histological Changes in the Labial Mucosa of SS Patients

Upon extensive observation of the whole labial tissues, edematous degeneration was found in the spinous layer of the epithelia in most tissues from the suspected SS patients, including those not eventually diagnosed as SS (Fig. 2). The degenerated cells had an enlarged or vacuolated appearance with weak eosin staining. Some nuclei were peripherally displaced or exhibited a ground-glass-like appearance. The arrangement of basal cells was irregular compared with that in mucous cysts. Some epithelia showed mild degeneration in the suprabasal layer with scattered islets of edematous cells covered by an apparently unimpaired layer. In cases with severe degeneration, cellular edema was distributed over the epithelia. No degeneration was found in the tissues from the mucous cyst patients. Among the 25 patients who underwent a labial biopsy, we observed severe degeneration (covering whole layers) in nine, mild degeneration (limited to the spinous layer) in 12, and no degeneration in four (Table 1). There were no significant correlations between presence of degeneration and age, salivary flow rate, or detection of anti-SS-A/Ro autoantibodies in serum among the suspected SS patients (data not shown). No bacteria were detected in the degenerated epithelia.

Impaired Arrangement of Adherens Junctional Molecules in Degenerated Epithelia

To characterize the epithelial degeneration and inflammatory infiltrates, we observed the expression of cell-cell adhesion molecules. In healthy mucosa, actin, E-cadherin, and β -catenin showed a net-like pattern delineating the plasma membrane (Fig. 3A, Supplemental Fig. 1B). Meanwhile, the degenerated region of the mucosa in SS patients exhibited weak or faint staining for F-actin, E-cadherin, and β -catenin with discontinuous patterns, suggesting disruption of cell-cell junctions. Iba1-positive cells were distributed around the edematous area (Fig. 3A). The number of intraepithelial Iba1-positive cells were significantly larger in the epithelia with edema than in those without edema (Fig. 3B). Adherens junctional proteins were reported to directly interact with TRPV4.¹⁴ TRPV4-immunoreactivity was weak in the degenerated region and scattered in the adjacent basal cell layers (Fig. 4A), while TRPV4-immunoreactivity in the healthy mucosa was clearly observed in the suprabasal layer.

(continued)

Figure 1. Immunofluorescence in human labial mucosa samples. Immune cells were immunostained for Iba1, CD11b, and CD207. (A) Iba1-positive cells in the labial mucosa of a mucous cyst patient and an SS patient. Images are shown with DIC. The dotted lines show the boundaries between epithelia and connective tissues. Numerous intraepithelial Iba1-positive cells are present in the SS mucosa. (B) Summary of the relationships between the number of intraepithelial Iba1-positive cells and the diagnosis. Greater numbers of inflammatory cells are observed in the epithelia from biopsy samples compared with the mucous cyst epithelia, despite the abundant presence of Iba1-positive cells in connective tissues in resected cyst samples with gross apparent inflammation. (C) Double staining for Iba1 and CD11b or CD207 in the labial mucosa from an SS patient. Iba1- or CD11b-positive cells have infiltrated not only the lamina propria, but also the epithelial layer. The middle row shows higher magnification images corresponding to the yellow square in the top row. Some Iba1-positive cells are strongly positive (thick arrows) or weakly positive (thin arrows) for CD11b. Some Iba1-positive and CD11b-negative cells and Iba1-negative and CD11b-positive cells are adjacent to one another in the connective tissue papillae (arrowheads). A few Iba1-positive cells are positive for CD207. (D) Summary of the numbers of intraepithelial Iba1-positive cells according to oral pain, oral dryness, and unstimulated salivary flow rate. Scale bars: 50 μ m. Abbreviations: Iba1, ionized calcium-binding adapter molecule 1; DAPI, 4',6-diamidino-2-phenylindole; DIC, differential interference contrast; SS, Sjögren's syndrome.

Figure 2. Representative photomicrographs of human labial mucosa from labial gland biopsy samples and resected mucous cyst samples as controls. The epithelium shows cellular edema, termed ballooning degeneration, mainly in the spinous layer. The lower row shows higher magnification images of the squares in the upper row. Edematous cells are larger in size, vacuolated, and weakly stained with eosin. Intercellular bridges are not apparent in the degenerated lesion. Some inflammatory cells are present in the lamina propria of the severely degenerated mucosa. Scale bars: 50 μ m.

TRPV4 Is Involved in Cell–Cell Adhesion of Oral Epithelial Cells

To clarify whether TRPV4 activation in oral epithelial cells contributes to the formation of intercellular contacts, we used a calcium switch model, in which

development of epithelial cell–cell contacts occurs in a calcium-dependent manner.²⁶ Primary oral epithelial cells were cultured in medium containing 0.07 mM CaCl_2 for 3 days to allow proliferation. After incubation for a further 24 hr in medium containing 0.07 mM (low) or 1.2 mM (high) CaCl_2 , the cells in the high-calcium

Figure 3. Immunofluorescence in human labial mucosa samples. (A) The dotted lines show the boundaries between epithelia and connective tissues. In the mucous cyst, the epithelium shows an organized arrangement of E-cadherin and β -catenin. In the SS mucosa, E-cadherin and β -catenin show discontinuous and irregular arrangements (arrowheads) with intraepithelial infiltration of Iba1- and CD11b-positive cells. (B) Summary of the relationships between the number of intraepithelial Iba1-positive cells and the presence of epithelial cellular edema. Scale bars: 50 μ m. Abbreviations: Iba1, ionized calcium-binding adapter molecule 1; SS, Sjögren's syndrome.

medium developed into a flat and continuous cellular sheet, while the cells in the low-calcium medium did not show clear cell–cell contacts (Fig. 4B). In the high-calcium medium, E-cadherin expression appeared as a thin linear staining at sites of cell–cell contacts in the cells from WT mice (Fig. 4B). However, in the cells from TRPV4KO mice, we found punctate E-cadherin expression with distinct intercellular gaps even in the high-calcium medium. The gaps were apparent as two peaks of fluorescence intensity. The effects of pharmacological TRPV4 activation were examined using the murine gingival epithelial cell line GE1 (Supplemental Fig. 1C). After incubation with TRPV4 agonist GSK1016790A (10 nM), the cells exhibited a clearer cell–cell contact line than control cells treated with dimethylsulfoxide. After treatment with TRPV4 antagonist GSK2193874 (1 μ M), E-cadherin labeling was discontinuous and had a broad width. These findings suggest that TRPV4 is involved in the assembly of intercellular adhesions between oral epithelial cells.

Discussion

Despite the growing interest in oral pain associated with local or systemic diseases, the mechanisms for

pain or dryness in the mouth remain unresolved. Recently, the epithelial barrier and immune cells were reported to affect skin hypersensitivity.²⁷ SS is a common disease accompanied by chronic hypersensitivity in the oral mucosa. To explore the relationship between oral pain and mucosal epithelia, we utilized labial tissue samples obtained during diagnostic biopsy procedures in SS patients, because it is not easy to collect tissue samples from patients experiencing pain only. We observed notable differences in the histological characteristics of mucosal epithelia samples between patients with and without pain.

The labial epithelia samples from patients with pain characteristically contained large numbers of Iba1- and CD11b-positive cells in the intercellular spaces adjacent to the epithelial edema. In particular, the number of Iba1-positive cells in the epithelia was greater in patients with pain than in patients without pain. The amoeboid-shaped cells observed in the degenerated mucosa with distinct Iba1 labeling appeared to resemble activated microglia. Because Iba1 is upregulated in activated macrophages/microglia and involved in pain signaling in nervous systems,²⁵ the increased number of intraepithelial Iba1-positive cells with activated morphological characteristics may

Figure 4. Immunofluorescence in human labial mucosa samples (A) and primary cultured mouse oral epithelial cells (B). (A) Expression of TRPV4 in a labial mucosa sample. In the unaffected area, TRPV4 shows distinct cytoplasmic expression in the spinous cells and weak membranous expression in the basal cells. In the degenerated area, TRPV4 expression is stronger in some basal cells and weaker in degenerated cells compared with the unaffected area. (B) After 24 hr of incubation in low-calcium medium, oral epithelial cells show apparent intercellular spaces and cytosolic E-cadherin and β -catenin. Meanwhile, after 24 hr of incubation in high-calcium medium, WT cells show cell-cell contacts with linear appearance of E-cadherin and β -catenin, while TRPV4KO cells show apparent intercellular gaps. The bottom row shows the fluorescence intensity profiles at the intercellular spaces indicated by the double-arrowhead yellow line. Scale bars: (A) 50 μ m, (B) 10 μ m. Abbreviations: SS, Sjögren's syndrome; TRPV, transient receptor potential channel vanilloid; WT, wild-type.

indicate reciprocal interplay among immune cells, epithelial cells, and peripheral nerves. We observed accumulation of Iba1- and CD11b-immunoreactive cells in the connective tissue papillae protruding toward the epithelia, suggesting that the immune cells were resident in or migrating from/to the epithelia. More CD11b-positive cells were localized along the

loops of blood vessels, suggesting that the cells moved from/into the vessels. We observed close topographical contacts between CD11b- and Iba1-positive cells, indicating reciprocal communications.²⁸ The topographical relationship among epithelia and inflammatory cells suggests their dynamic interplay via cytokines.

Upon extensive histological observation, we noted cellular edema with macrophage infiltration in most of the labial epithelial sections from SS patients. Cellular edema is defined as ballooning degeneration in the skin,²⁹ and is associated with atopic dermatitis or herpes infections.^{30,31} To our knowledge, there are few reports demonstrating ballooning degeneration in the oral mucosa of SS patients, although it is possible that pathologists may notice these changes during the process of diagnostic observations. Initially, we suspected that the degeneration was caused by hyposalivation. Unexpectedly, however, we did not find a significant correlation between edema and salivary flow. Furthermore, the area of degeneration was found as distributed islets of edematous cells in the spinous layer, rather than in the superficial epithelia facing the oral cavity. Thus, we suggest that hyposalivation and dryness from the external environment are not the leading causes of the ballooning degeneration. Interestingly, macrophage infiltrates were observed around and adjacent to the edematous epithelia. It is suggested that these cells may have an active role in the degenerative processes with pathological and/or reparative properties. Further studies are needed to characterize the properties of these epithelial macrophages. We consider that degeneration with presence of macrophages in the epithelia may be one of the characteristics of SS, although it is not a specific finding.

The epithelium serves as an important barrier via intimate intercellular connections with junctional complexes. In normal epithelia, adherens junctional proteins such as E-cadherin, β -catenin, and F-actin showed mesh-like arrangements that delineated the surface plasma membranes of epithelial cells, similar to the observations in the normal labial epithelia. Meanwhile, in the edematous region of the epithelia, faint or irregular distributions of E-cadherin and F-actin were observed. Disruption of membranous E-cadherin is associated with impaired epithelial barrier functions,³² such as those observed in skin with atopic dermatitis or bronchial epithelia with asthma.^{6,33,34} In SS patients, elevated gene expression of inflammatory cytokines was demonstrated in the salivary glands and secreted saliva.^{35,36} Furthermore, recent LC-MS (liquid chromatography–mass spectrometry) analyses of whole saliva from SS patients revealed upregulated pathways related to calcium binding, actin disassembly, and cell adhesion or membrane fusion.³⁷ The degenerated epithelia with activated immune cells could result from environmental factors such as viruses and other activators of innate immunity or inflammatory milieu with infiltrating cells. There is accumulating evidence that inflammatory cytokines like $\text{INF-}\gamma$ (interferon gamma) and $\text{TNF-}\alpha$ (tumor necrosis factor- α) disrupt the adherens junctional structures of E-cadherin in the

mucosa.³⁸ Taken together, not only the components of adaptive immunity but also the cellular junctions of epithelia could be potential targets for treatment of SS.

The epithelia showing disarrangement of intercellular E-cadherin were devoid of TRPV4-immunoreactivity. TRPV4 was reported to play a key role in adherens junctions through direct interactions with β -catenin, E-cadherin, and actin.^{14,39,40} Our results showed that degeneration of the labial epithelia was accompanied by impairment of adherens junctions. We also confirmed that TRPV4 contributed to intercellular junctions in oral epithelia using primary cultured oral epithelial cells from mice with and without TRPV4 gene deficiency. Considering that TRPV4 expression was distinct in the spinous layer of the normal labial epithelia, and weak and ambiguous in the degenerated area, edematous degeneration may be due to incomplete integration of adherens junctional complexes and TRPV4.

Recently, epithelial cells have been considered sentinels for inner and outer environmental changes.⁴¹ Epithelial TRPV4 was proposed as one of the sensory molecules. In esophageal epithelia, TRPV4 activation by chemical or mechanical stimuli was shown to release the pain transmitter ATP.⁴² Skin keratinocytes released endothelin through TRPV4 after UV-B radiation, leading to pain behaviors in mice.⁴³ We previously identified TRPV4 as a warm sensor in oral epithelial cells.^{12,13} Thus, TRPV4 may play a role in changes to the human lips, which are constantly exposed to thermal or mechanical stimuli. Furthermore, because we found a significant correlation between pain manifestation and inflammatory cell infiltration within the degenerated epithelia, dysregulation of TRPV4 in the labial epithelium may affect oral mucosal sensitivity.

In summary, we have revealed relationships among oral pain, intraepithelial infiltrating macrophages, and epithelial barrier impairment. As several small molecules have been identified for TRPV4 pharmacology,^{15,44,45} activation of TRPV4 in the oral epithelium is one of the options for treatment for oral disorders involving pain.

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Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

RUY and MAK contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; RA, Yukiko O, JY, TK, Yasuyoshi O, A-LC, AD, and YY contributed to data acquisition and analysis, and critically revised the manuscript. TK performed histopathological diagnoses and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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