Journal of
PERIODONTAL RESEARCH

J Periodont Res 2010; 45: 579–582

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2009.01258.x

Short communication

# The expressions of claudin-1 and E-cadherin in junctional epithelium

**T. Fujita, K. Hayashida, H. Shiba, A. Kishimoto, S. Matsuda, K. Takeda, H. Kawaguchi, H. Kurihara**

Department of Periodontal Medicine, Division of Frontier Medical Science, Hiroshima University Graduate School of Biomedical Sciences, Kasumi, Minami-ku, Hiroshima, Japan

*Fujita T, Hayashida K, Shiba H, Kishimoto A, Matsuda S, Takeda K, Kawaguchi H, Kurihara H. The expressions of claudin-1 and E-cadherin in junctional epithelium. J Periodont Res 2010; 45: 579–582. © 2010 John Wiley & Sons A/S*

*Background and Objective:* The epithelium provides an important barrier against microbial invasion. Tight junction structural proteins called claudins are known to contribute to the epithelial cell barrier. Junctional epithelium is located at a strategically important interface between gingival sulcus and is interconnected by desmosomes and gap junctions, but not by tight junctions. Although claudins are tight junction-associated proteins, they are also expressed in the epithelium despite its lack of tight junctions in invertebrates. Therefore, claudins may play an important role in junctional epithelium without tight junctions. E-cadherin is a key molecule in the formation of adherence junctions and desmosomes. In the present study, we aimed to investigate the expressions of claudin-1,claudin-3, claudin-7 and E-cadherin in the junctional epithelium of Fischer 344 rats.

*Material and Methods:* Gingival tissues from Fischer 344 rats were analyzed by immunohistochemical staining for claudin-1, claudin-3, claudin-7, and E-cadherin.

*Results:* Intense staining for claudin-1 and E-cadherin were observed in the junctional epithelium. In contrast to claudin-1, claudin-3 was mainly expressed in oral gingival epithelium and claudin-7 could not be detected on immunohistochemical analysis of the rat gingiva.

*Conclusion:* These data suggest that claudin-1 and E-cadherin exist in the junctional epithelium and may play an important role in epithelial barrier function.

Tsuyoshi Fujita DDS, PhD, Department of Periodontal Medicine, Division of Frontier Medical Science, Hiroshima University Graduate School of Biomedical Sciences, 1-2-3, Kasumi, Minami-ku, Hiroshima 734 8553, Japan
Tel: +81 82 257 5663
Fax: +81 82 257 5664
e-mail: tfuji@hiroshima-u.ac.jp

Key words: claudin-1; E-cadherin; junctional epithelium; oral gingival epithelium

Accepted for publication October 4, 2009

The epithelium provides an important barrier against microbial invasion. Epithelial cells are generally interconnected by tight junctions, adherens junctions, desmosomes and gap junctions. The junctional epithelium is located at a strategically important interface at the base of the gingival sulcus and contributes activately to inflammatory processes, since it represents the first line of defense against microbial attack (1–3). Previous studies have shown that the junctional epithelium is interconnected by a only few desmosomes and occasional gap junctions and shows wide intercellular spaces (2–6). Therefore, it is unlikely that tight junctions contribute to the barrier function of the junctional epithelium (4), although they are essential for the tight sealing of cellular sheets, thus controlling paracellular ion flux and maintaining tissue homeostasis (7,8).

Claudins are tight junction-associated tetraspan proteins with relatively short cytoplasmic amino- and carboxy-termini flanking a first extracellular loop of 53 amino acids and a second shorter loop of 24 amino acids (7). Claudins are expressed in invertebrates despite their absence of tight junctions (9). The epithelial barriers of invertebrates are formed by septate junctions with wide intercellular gaps, very different from the near fusions at tight junctions. Claudin-1-deficient mice died within 1 d of birth and showed severe defects in the permeability of the epidermis (10). Cells overexpressing claudin-1, claudin-3 or claudin-7 showed increased transepithelial electrical resistance (11–13). Therefore, claudins are thought to play an important role in regulating the epithelial

barrier at tight junctions and septate junctions (14). In spite of the absence of tight junctions, claudins may contribute to the epithelial barrier in junctional epithelium; however, the protein and mRNA expressions of claudins in healthy junctional epithelium have not been investigated. In addition, an immunohistochemical study revealed that claudin-1 was not detected in the junctional epithelium from adult patients with periodontitis (15). Therefore, the expression of claudin-1 in healthy junctional epithelium has not been clarified. E-Cadherin, which is a key molecule in the formation of adherens junctions and desmosomes, plays a crucial role in maintaining the structural integrity (16). In previous studies, the expression of E-cadherin was detectable in normal junctional epithelium, although there was a striking reduction or no reaction in staining for E-cadherin in diseased junctional epithelium (15,17). Therefore, it may be considered that E-cadherin does not function in diseased junctional epithelium. To investigate the expression of claudin-1, caludin-3, claudin-7 and E-cadherin in healthy junctional epithelium, we examined it immunohistochemically using Fischer 344 rats.

## Material and methods

### Animal experimentation

After obtaining approval from the animal care committee of Hiroshima University, a total of twenty 9-wk-old male Fischer 344 rats were used in this study. Under general anaesthesia induced by intraperitoneal injection of 20% ethyl carbamate (30 mg/kg body weight). Rats were killed with an overdose of ethyl ether.

Tissue samples were resected *en bloc* from the left and right molar regions and fixed with 4% paraformaldehyde solution. They were then decalcified in a 10% ethylenediaminetetraacetic acid (EDTA) solution in phosphate-buffered saline (PBS) for 14 d at 4°C. The decalcified tissue blocks were embedded in paraffin. Sections (5 μm thick) of the frontal plane parallel to the long axis of the tooth, including the root apex, were cut and collected on glass slides.

### Immunohistochemistry

After deparaffinization, the endogenous horseradish peroxidase was deactivated with 3% hydrogen peroxide in PBS. Then, each section was incubated with 0.2% casein in Tris–HCl buffered saline (TBS, 20 mM Tris–HCl, 0.15 M NaCl, pH 7.6) for 30 min at room temperature and then with rabbit anti-human claudin-1, claudin-3, claudin-7 (Thermo Scientific, Fremont, CA, USA) and goat anti-mouse E-cadherin (R&D Systems, Minneapolis, MN, USA) for 24 h at 4°C. The species reactivities of rabbit anti-human claudin-1 and claudin-3 to rat were previously tested (Thermo Scientific). After being rinsed with PBS, the sections were incubated with biotinylated anti-rabbit immunoglobulin G or anti-goat immunoglobulin G for 30 min. The sections were rinsed with PBS, incubated with peroxidase-conjugated streptavidin for 30 min, and then rinsed with PBS. The colour was developed with 0.025% 3,3′-diaminobenzidine tetrahydrochloride in TBS plus hydrogen peroxide. The specimens were counterstained with methyl green, dehydrated, mounted, observed by light microscopy (Eclipse E600; Nikon, Tokyo, Japan), and then photographed using a digital camera (DXM1200, Nikon) and imaging software (ACT-1, Nikon). Negative control samples were prepared by replacing the anti-rabbit immunoglobulin G or anti-goat immunoglobulin G with normal rabbit immunoglobulin G or normal goat immunoglobulin G. Twenty samples from 20 rats were examined by immunohistochemistry for each antibody. All samples showed similar reactions for each antibody. Each panel in Fig. 1 represents the findings from 20 samples.

## Results

Figure 1 shows a representative haematoxylin and eosin stained section (Fig. 1A) and immunohistochemical study of gingiva for claudin-1, claudin-3, claudin-7 and E-cadherin in Fischer 344 rats. Intense staining of claudin-1 in the junctional epithelium at cell–cell contact sites was noted (Fig. 1B,C). In contrast to claudin-1, strong staining for claudin-3 occurred in the granular layer and spinous layer of the oral gingival epithelium, but there was much less intense staining in the junctional epithelium and sulcus epithelium (Fig. 1D,E). Claudin-7 was not detected on immunohistochemical analysis of the rat gingiva (Fig. 1F). The inset in Fig. 1F indicates the positive reaction for claudin-7 antibody in rat oral mucosal epithelium. Furthermore, E-cadherin was expressed in the junctional epithelium, sulcus epithelium and oral epithelium at the cell–cell junctions in the basal and suprabasal layers (Fig. 1G–I). Non-specific staining was not observed in negative control samples.

## Discussion

In this study, we have demonstrated, for the first time, that claudin-1 is present in junctional epithelium of Fischer 344 rats. Claudin-1 is known to be a major structural protein of tight junctions. However, since tight junctions in junctional epithelium are not well developed and are either discontinuous or very thin, formed by a few strands (4), claudin-1 seems not to form tight junctions in the junctional epithelium. In spite of being a tight junction-associated protein, claudin-1 is also expressed in epithelium lacking tight junctions. In invertebrates, septate junctions circumscribe epithelial cells and have been regarded as the functional counterparts of tight junctions (14). *Drosophila* exhibits six claudin sequences, and one of them, Megatrachea, is located at septate junctions (14). Mutations of Megatrachea disrupt the barrier and result in defects of the size and shape of the tracheal epithelium (18). In addition, claudin-1-expressing cells exhibit higher transepithelial electrical resistance than wild-type Madin–Darby canine kidney cells, and show reduced paracellular flux (11). These results imply that claudin-1 contributes to the epithelial barrier in junctional epithe-

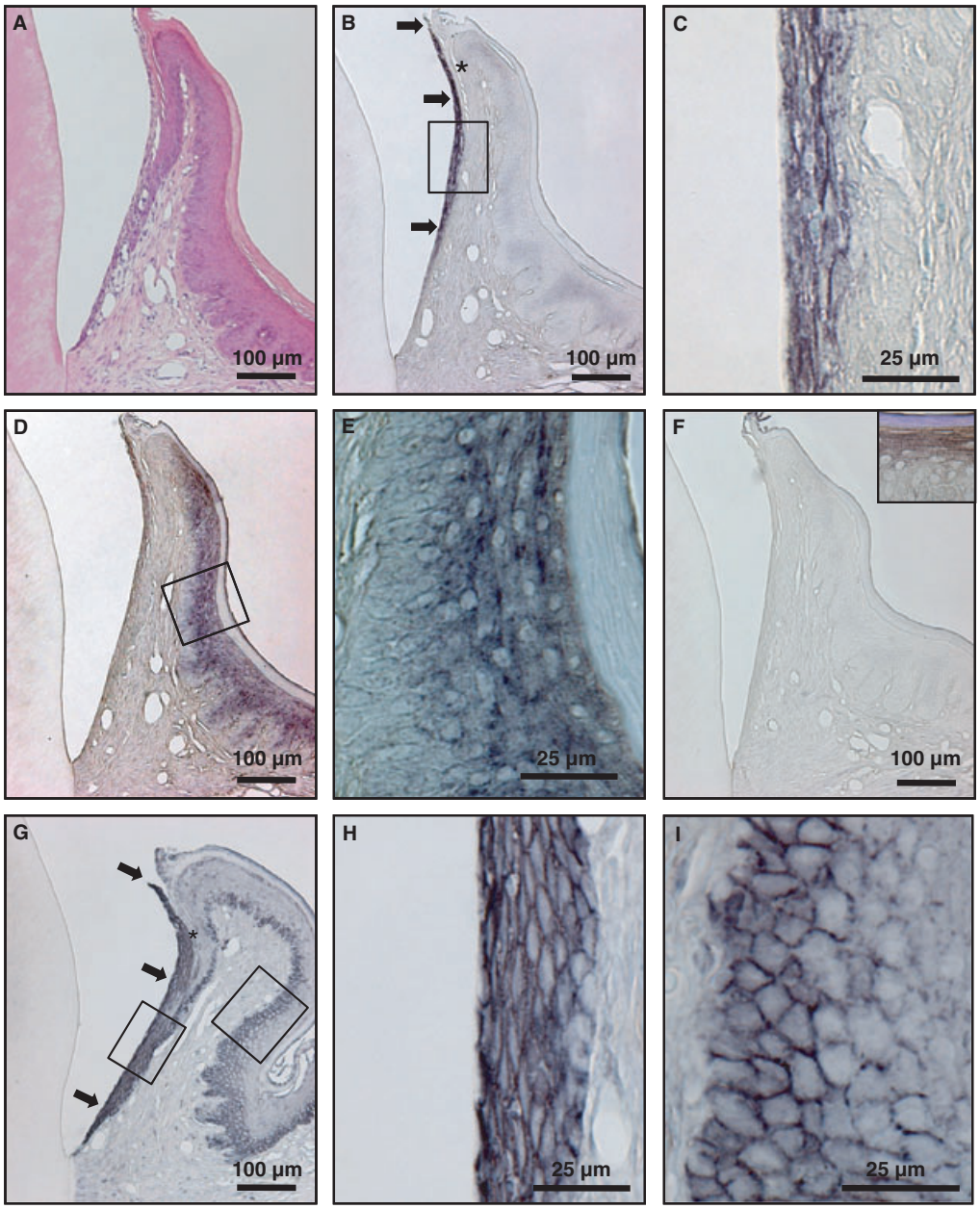


*Fig. 1.* Immunohistochemical staining of claudin-1, claudin-3, claudin-7 and E-cadherin in gingival tissues of rats. (A) Haematoxylin and eosin staining. (B,C) Immunolocalization of claudin-1. Arrows indicate junctional epithelium and asterisk shows sulcus epithelium. (C) Higher magnification of boxed area in (B). (D,E) Immunolocalization of claudin-3. (E) Higher magnification of boxed area in (D). (F) Immunolocalization of claudin-7. A positive control section is shown in the inset. (G,H,I) Immunolocalization of E-cadherin. Arrows indicate junctional epithelium and asterisk shows sulcus epithelium. (A,B,D,F,G) Original magnification ×200. (C,E) Original magnification ×400. (H) Higher magnification of boxed area on left side in (G) (×400). (I) Higher magnification of boxed area on right side in (G) (×400).

lium. In addition, claudin-1 regulates epithelial–mesenchymal transformation through Wnt/β catenin signalling in human cancer cell lines (19). Therefore, claudin-1 may exhibit signalling as well as barrier functions. Further studies to investigate the role of claudin-1 in junctional epithelium are required.

In the present study, claudin-1 was detected in the junctional epithelium from Fischer 344 rats, although Hatakeyama *et al.* (15) reported that claudin-1 was not expressed in human diseased junctional epithelium from patients with periodontitis. We used healthy Fischer 344 rats maintained in specific pathogen-free conditions in this experiment, and claudin-1 was detected in our immunohistochemical study. In contrast, Hatakeyama *et al.* (15) found claudin-1 in the uppermost layers of human oral epithelium, whereas claudin-1 was not detected in the oral epithelium in the present study. The difference in claudin-1 expression between the previous and

present studies might be due to anatomical differences or species differences between humans and rats.

In contrast to claudin-1, claudin-3 was mainly expressed in oral gingival epithelium and claudin-7 could not be detected on immunohistochemical analysis of the rat gingiva. Thus, although claudin-1, claudin-3 and claudin-7 are involved in enhancement of the barrier function in epithelial cells (11–13), the expression pattern of the claudins in the gingiva is different. These data are similar to the previous report that claudins-1, -3, -5, -6, -11 and -18 are expressed in different pattern in the differentiated and/or undifferentiated compartments of the epidermis and nail, tongue, oral mucosa and stomach of the mouse (20). The claudin family exhibits distinct expression patterns in tissue- and cell-type-specific manners (14,21). The differences in expression and localization of claudins in the various gingival epithelial tissues probably contribute to their demonstrated differences in paracellular transport and permeability in each tissue and tissue compartment. The junctions with different claudin combinations differ from each other in ion and solute selectively.

In the present study, E-cadherin was expressed in the junctional epithelium, sulcus gingival epithelium and oral gingival epithelium. Our findings confirm the localization of E-cadherin in healthy gingiva, as previously reported (17). E-cadherin is a key molecule to protect against bacterial invasion in junctional epithelium, since junctional epithelial cells are mainly interconnected by desmosomes. In addition, E-cadherin was expressed in far fewer cells in diseased junctional epithelium than in healthy junctional epithelium (17), or was not detectable in the diseased junctional epithelium from patients of periodontitis (15). This may be similar to the claudin-1 expression pattern in the healthy and diseased junctional epithelium. These data imply that claudin-1 as well as E-cadherin may contribute to epithelial permeability in healthy junctional epithelium.

It has been thought that the barrier function of junctional epithelium is related to the outward flow of gingival fluid and the transmigration of neutrophilic granulocytes between epithelial cells (3). Furthermore, antimicrobial peptides called defensins play an important role in inflammatory stimulation (22). The regulation of the expression in claudin-1 and E-cadherin may be a new candidate for the prevention of periodontal disease.

## Acknowledgements

This study was supported in part by a grant-in-aid for the encouragement of young scientists (B) (no. 20791613) from the Japan Society for the Promotion of Science, Japan.

## References

1. Schroeder HE, Listgarten MA. The junctional epithelium: from strength to defense. *J Dent Res* 2003;**82:**158–161.
2. Bosshardt DD, Lang NP. The junctional epithelium: from health to disease. *J Dent Res* 2005;**84:**9–20.
3. Shimono M, Ishikawa T, Enokiya Y *et al.* Biological characteristics of the junctional epithelium. *J Electron Microsc (Tokyo)* 2003;**52:**627–639.
4. Saito I, Watanabe O, Kawahara H, Igarashi Y, Yamamura T, Shimono M. Intercellular junctions and the permeability barrier in the junctional epithelium. A study with freeze-fracture and thin sectioning. *J Periodontal Res* 1981;**16:**467–480.
5. Schroeder HE, Munzel-Pedrazzoli S. Morphometric analysis comparing junctional and oral epithelium of normal human gingiva. *Helv Odontol Acta* 1970;**14:**53–66.
6. Schroeder HE. Ultrastructure of the junctional epithelium of the human gingiva. *Helv Odontol Acta* 1969;**13:**65–83.
7. Schneeberger EE, Lynch RD. The tight junction: a multifunctional complex. *Am J Physiol Cell Physiol* 2004;**286:**C1213–C1228.
8. Gonzalez-Mariscal L, Betanzos A, Nava P, Jaramillo BE. Tight junction proteins. *Prog Biophys Mol Biol* 2003;**81:**1–44.
9. Van Itallie CM, Anderson JM. Claudins and epithelial paracellular transport. *Annu Rev Physiol* 2006;**68:**403–429.
10. Furuse M, Hata M, Furuse K *et al.* Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol* 2002;**156:**1099–1111.
11. Inai T, Kobayashi J, Shibata Y. Claudin-1 contributes to the epithelial barrier function in MDCK cells. *Eur J Cell Biol* 1999;**78:**849–855.
12. Hashimoto K, Oshima T, Tomita T *et al.* Oxidative stress induces gastric epithelial permeability through claudin-3. *Biochem Biophys Res Commun* 2008;**376:**154–157.
13. Alexandre MD, Lu Q, Chen YH. Overexpression of claudin-7 decreases the paracellular Cl- conductance and increases the paracellular Na+ conductance in LLC-PK1 cells. *J Cell Sci* 2005;**118:**2683–2693.
14. Furuse M, Tsukita S. Claudins in occluding junctions of humans and flies. *Trends Cell Biol* 2006;**16:**181–188.
15. Hatakeyama S, Yaegashi T, Oikawa Y *et al.* Expression pattern of adhesion molecules in junctional epithelium differs from that in other gingival epithelia. *J Periodontal Res* 2006;**41:**322–328.
16. Wheelock MJ, Jensen PJ. Regulation of keratinocyte intercellular junction organization and epidermal morphogenesis by E-cadherin. *J Cell Biol* 1992;**117:**415–425.
17. Ye P, Chapple CC, Kumar RK, Hunter N. Expression patterns of E-cadherin, involucrin, and connexin gap junction proteins in the lining epithelia of inflamed gingiva. *J Pathol* 2000;**192:**58–66.
18. Behr M, Riedel D, Schuh R. The claudin-like megatrachea is essential in septate junctions for the epithelial barrier function in Drosophila. *Dev Cell* 2003;**5:**611–620.
19. Dhawan P, Singh AB, Deane NG *et al.* Claudin-1 regulates cellular transformation and metastatic behavior in colon cancer. *J Clin Invest* 2005;**115:**1765–1776.
20. Troy TC, Arabzadeh A, Yerlikaya S, Turksen K. Claudin immunolocalization in neonatal mouse epithelial tissues. *Cell Tissue Res* 2007;**330:**381–388.
21. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol* 2001;**2:**285–293.
22. Chung WO, Dommisch H, Yin L, Dale BA. Expression of defensins in gingiva and their role in periodontal health and disease. *Curr Pharm Des* 2007;**13:**3073–3083.