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ADAM17 regulates TNF-α expression upon lipopolysaccharide stimulation in oral keratinocytes

Aya HIRAYAMA[1], Shuji AWANO[2], Yuji SETA[3], and Toshihiro ANSAI[1]

[1] Division of Community Oral Health Development, Kyushu Dental University, 2-6-1 Kokurakita-ku, Kitakyushu 803-8580, Japan, [2] Department of Clinical Education Development and Research, Kyushu Dental University, 2-6-1 Kokurakita-ku, Kitakyushu 803-8580 and [3] Department of Anatomy, Kyushu Dental University, 2-6-1 Kokurakita-ku, Kitakyushu 803-8580, Japan

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ABSTRACT

A disintegrin and metalloprotease 17 (ADAM17) is a tumor necrosis factor (TNF)-converting enzyme and was first identified as the enzyme that cleaves the prodomain of TNF-α, a proinflammatory cytokine that plays a central role in immune regulation and a variety of inflammatory responses in destructive periodontal disease. The aim of the present study was to verify the presence of ADAM17 in the gingival epithelium and elucidate its involvement in the release of TNF-α in oral keratinocytes. Immunohistochemical analyses of ADAM17 were performed in gingival tissues obtained from patients and in human oral keratinocytes (HOKs). Additionally, levels of TNF-α and ADAM17 in HOKs exposed to lipopolysaccharide (LPS) were assessed using enzyme-linked immunosorbent assays. Moreover, the effects of ADAM17 inhibitor, matrix metalloproteinase (MMP) inhibitor, and ADAM17 siRNA on TNF-α concentration were assessed. Strong immunoreactivity for ADAM17 was observed in the epithelium of the inflamed gingival tissues and in HOKs. Furthermore, treatment with either ADAM17 inhibitor or ADAM17 siRNA inhibited the generation of TNF-α induced by LPS in HOKs. The present study demonstrates that ADAM17 is strongly expressed in the epithelium of gingival tissues and suggests that ADAM17 may be a key enzyme that regulates the generation of TNF-α in oral keratinocytes.

Periodontitis is an inflammatory disease that destroys the supporting osseous and connective tissues of the teeth, and is the most common cause of tooth loss in adults (21). The periodontal inflammatory process involves various proinflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α), which are considered important modulators of inflammation (1, 2, 4). TNF-α is a pro-inflammatory cytokine that plays a central role in immune regulation and in a variety of inflammatory responses during destructive periodontal disease, thus contributing to the onset of periodontal inflammation (12).

A disintegrin and metalloprotease 17 (ADAM17) is a member of the ADAM family, a group of zinc-dependent proteases that cleave membrane-bound proteins and/or degrade the extracellular matrix (7). ADAM17 is a TNF-converting enzyme, first identified as the enzyme that cleaves the prodomain of TNF-α, and is thought to be the main protease responsible for the release of TNF-α during the inflammatory response (3, 16, 17). In fact, the enzymatic activity of ADAM17 is enhanced in diseases such as rheumatoid arthritis and inflammatory bowel disease, which are characterized by elevated levels of circulating or tissue TNF-α (8, 16, 18).

We recently reported that the severity of periodontal disease might be associated with expression

Address correspondence to: Dr. Shuji Awano
Department of Clinical Education Development and Research, School of Dentistry, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu 803-8580, Japan
Tel: +81-93-285-3119
E-mail: [redacted]

of the ADAM17 gene in the buccal mucosal epithelium (15). However, details of the ADAM family role in the oral cavity, including that of ADAM17 in periodontal disease, remain unclear. We hypothesized that the ADAM17 in oral mucosal epithelium would be associated with development of oral diseases such as periodontitis. Therefore, the present study aimed to verify the presence of ADAM17 in the gingival epithelium and elucidate the involvement of ADAM17 in TNF-α expression in oral keratinocytes during the inflammatory response.

MATERIALS AND METHODS

Tissue preparation. This study was conducted with the approval of the Kyushu Dental University Research Ethics Committee (approval no. 010-032). Informed written consent was obtained from the patients.

Gingival tissues were obtained from two male patients aged 60 and 65 years, who were attending the hospital at Kyushu Dental University for treatment of chronic periodontitis. Neither patient had any systemic disorders. The gingival tissues were obtained from sites at a periodontal probing depth of ≥ 5 mm during 'bleeding on probing' periodontal treatment. The sample tissues were immediately frozen and stored at −80°C.

Cell culture of human oral keratinocytes. Human oral keratinocytes (HOKs, #2610) originating from the oral mucosa and human oral mucosal keratinocyte 107 cells (hOMK107, #HOMK107) originating from the gingival mucosa were purchased from ScienCell Research Laboratories (Carlsbad, CA, USA) and CellResearch Corporation (Singapore), respectively. Oral Keratinocyte Medium-New Zealand Origin BPE (OKM-NZ), Oral Keratinocyte Growth Supplement-New Zealand Origin BPE (OKGS-NZ), and penicillin/streptomycin solution (P/S) were purchased from ScienCell Research Laboratories. Keratinocytes were cultured in a keratinocyte growth medium consisting of OKM-NZ with OKGS-NZ and P/S (100 units/mL of Penicillin and 100 μg/mL of Streptomycin). Cells were grown in 5% CO_2 at 37°C.

Reagents. Lipopolysaccharide (LPS) from *Escherichia coli (E. coli)* and *Porphyromonas gingivalis* (*P. gingivalis*) were used as inflammation-inducers and were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Astarte Biologics (Bothell, WA, USA), respectively. TNF-α processing inhibitor-0 (TAPI-0), a TNF-α converting enzyme/ADAM17 inhibitor, and GM1489, a potent broad-range inhibitor of matrix metalloproteinases (MMPs), were purchased from Merck Millipore (Tokyo, Japan). Rabbit polyclonal antibody against ADAM17, CF™555-labeled goat anti-rabbit IgG, rabbit polyclonal IgG antibodies, and a horseradish-peroxidase (HRP)-conjugated anti-mouse IgG were purchased from Eno-Gene Biotech Co. (New York, NY, USA), Biotium, Inc. (Hayward, CA, USA), Abcam (Tokyo, Japan), and GE Healthcare (Tokyo, Japan), respectively. VECTASHIELD Mounting Medium with DAPI was purchased from Vector Laboratories, Inc. (Burlingame, CA, USA). Phosphate-buffered saline (PBS), anti-β-actin monoclonal antibody and a HRP-conjugated anti-rabbit IgG were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Small interfering RNA (siRNA) targeting ADAM17, siRNA transfection reagent, siRNA transfection medium, siRNA dilution buffer, and scrambled control siRNA were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Immunoblotting. HOKs and hOMK107 cells were grown to near confluence in 6-well culture plates (Iwaki® Cell Biology, Tokyo, Japan) and lysed with lysis buffer (50 mM Tris/HCl buffer pH 7.5, 100 mM NaCl, 1 mM EDTA and 1% TritonX100). The lysate samples were fractionated on 8% SDS-PAGE and transferred to PVDF transfer membranes (Hybond-P; GE Healthcare, Tokyo, Japan). Membranes were probed with anti-ADAM17 polyclonal antibody (concentration, 1 : 1000) or anti-β-actin monoclonal antibody (1 : 500). Membranes were incubated with an HRP-conjugated anti-rabbit IgG (1 : 10,000), or a HRP-conjugated anti-mouse IgG (1 : 2,000) as a second antibody. After additional washes, bound antibodies were detected using ECL reagents (GE Healthcare).

Immunofluorescence analyses of ADAM17. Tissue fragments were fixed in 4% paraformaldehyde, embedded in paraffin and sectioned (5 μm). Sections were then deparaffinized by immersion in xylene and a graded series of ethanol (70–100%). The tissue sections were treated for 30 min in blocking buffer (Tris-buffered saline, 1% bovine serum, and 0.2% gelatin) and incubated with rabbit polyclonal ADAM17 antibody (working dilution, 1 : 200) overnight at room temperature. After rinsing in PBS, the sections were incubated with CF™555 goat anti-rabbit IgG (working dilution, 1 : 1000) overnight at room temperature in a dark room. After rinsing the

sections in PBS, VECTASHIELD Mounting Medium with DAPI was added.

HOKs and hOMK107 cells were grown to 60% confluence on a CHAMBER SLIDE II (Iwaki® Cell Biology), washed three times, then fixed and permeabilized for 15 min in methanol/acetone (1 : 1) at −20°C. The cells on cover slips were treated for 30 min in blocking buffer and labeled with anti-ADAM17 primary antibodies (1 : 200). The cells were washed three times in PBS and incubated overnight at room temperature with CF™555 goat anti-rabbit IgG (1 : 1000). The cells on cover slips were washed an additional three times in PBS and stained using VECTASHIELD Mounting Medium with DAPI.

All preparations were visualized using a fluorescence microscope (BZ-9000; KEYENCE, Osaka, Japan). Images were captured digitally and processed using BZ-II imaging software (KEYENCE).

For the negative control, the primary antibody was replaced with the primary antibody diluent/ blocking buffer or isotype control/ rabbit polyclonal IgG (1 : 1000). Controls were negative in all cases.

Cell viability. HOKs were seeded at 5.0×10^3 cells per well in 96-well culture plates (Iwaki® Cell Biology). HOKs viability following exposure to *E. coli* or *P. gingivalis* LPS, TAPI-0, or GM1489, was compared with control cell viability using the CellQuanti-MTT Cell Viability Assay kit (BioAssay Systems, Hayward, CA, USA), following the manufacturer's protocol.

Enzyme-linked immunosorbent assay (ELISA) to detect active ADAM17 and TNF-α from HOKs. HOKs were seeded at 1.0×10^5 cells per well in 24-well culture plates (Iwaki® Cell Biology). After 24 h, the cells were washed once in PBS and incubated for a further 3, 6, or 12 h in medium containing 1 or 10 μg/mL of LPS from *E. coli* or *P. gingivalis*, or in medium alone (control).

HOKs seeded in the same way as those described above were incubated for a further 6 h in medium containing 10 μg/mL LPS from *E. coli* or *P. gingivalis* alone or in combination with either 100 nM TAPI-0, 500 nM GM1489, or 100 nM TAPI-0 plus 500 nM GM1489; or in medium alone (control).

The concentrations of ADAM17 in the cell lysates and TNF-α in the cell culture supernatants were determined using the ELISA Kit for ADAM17 (Sensitivity: 0.060 ng/mL; Cloud-Clone Corp., Houston, TX, USA) and the Human TNFα ELISA Kit (Sensitivity: 8 pg/mL; Diaclone, Besancon Cedex, France), respectively, according to the manufacturers' protocols.

RNA interference. HOKs were seeded in a CHAMBER SLIDE II and at 5.0×10^3 cells per well in 96-well culture plates, and siRNA-targeting ADAM17 was then transfected into the cells according to the manufacturer's instructions. Negative control cells were exposed to scrambled siRNA oligonucleotide. After transfection, cells in the CHAMBER SLIDE II were cultured in keratinocyte growth medium for 24, 48, or 72 h. Down-regulation of ADAM17 protein was then confirmed using immunofluorescence as described above. Cells randomly selected after transfection were normalized to the number of cell nuclei in each image with a minimum of 100 cells, and the mean fluorescence intensity for ADAM17 was measured using BZ-II imaging software (KEYENCE).

Cells in the 96-well culture plates were incubated for 24, 48, and 72 h in serum-free keratinocyte growth medium following transfection. Viability of these cells was analyzed as described above.

Cells were also seeded at 1.7×10^5 cells per well in 24-well culture plates. At 72 h after transfection with siRNA-targeting ADAM17 and the scrambled siRNA oligonucleotide, the cells were incubated for 6 h in medium containing 10 μg/mL LPS from *E. coli* or medium alone (control), and the concentration of TNF-α in the cell culture supernatant was analyzed as described above.

Statistical analyses. Data are expressed as the mean ± standard deviation for double or triple determinations. Statistical significance between untreated controls and each sample was determined by analysis of variance (ANOVA) with Dunnett's test and the *t*-test. Correlations between the ADAM17 and TNF-α levels in HOKs were assessed by Spearman's rank correlation analysis. All analyses were conducted using SPSS software (IBM SPSS Statistics, version 20-J; IBM Corp., Armonk, NY, USA). The level of statistical significance was set at <0.05.

RESULTS

Expression of ADAM17 in gingival tissues and HOKs

Immunohistochemical analysis was performed to verify ADAM17 protein expression in the gingival epithelium (Fig. 1). The ADAM17 immunoreaction showed the coarse granular cytoplasmic staining pattern in the cells of the gingival epithelium. Furthermore, immunoblotting and immunofluorescence

analyses were performed to examine the protein expression and differences in subcellular distribution of ADAM17 in HOKs and hOMK107 cells (Fig. 2).

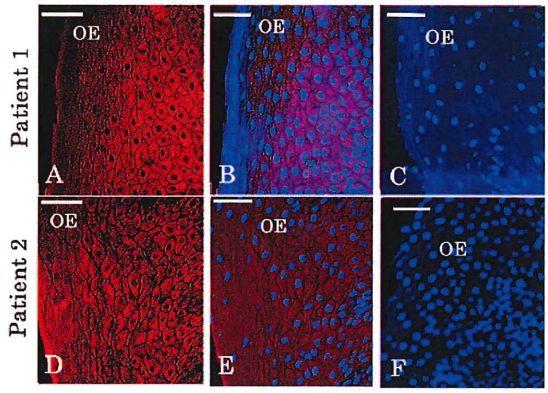

Fig. 1 Immunofluorescence of ADAM17 in gingival tissues. Microscopy images of ADAM17 (red) (A and D); ADAM17 (red) counterstained with nuclear DAPI (blue) (B and E); and isotype control counterstained with nuclear DAPI (blue) (C and F). A–C and D–F show the images of gingival tissues obtained from patient 1 and 2, respectively. OE: oral epithelium. White bar, 50 μm.

By immunoblotting analysis, two characteristic immunoreactive protein bands, corresponding to the 120 kDa immature form and 100 kDa mature form of ADAM17, were consistently detected in the blots of lysates of HOKs and hOMK107 cells (Fig. 2A). Additionally, ADAM17 was expressed at high levels in the cytoplasm of HOKs and hOMK107 cells, with especially high fluorescence observed in the nucleus of HOKs and the perinuclear region of hOMK107 cells (Fig. 2B–G).

Effect of LPS, TAPI-0, and GM1489 on cell viability

The viability of HOKs treated with inflammatory inducers (LPS of *E-coli* and *P. gingivalis*), MMP inhibitor (GM1489), and ADAM17 inhibitor (TAPI-0) was assessed. None of the reagents added to the cultured cells had any influence on cell viability.

Effect of LPS on protein expression levels of TNF-α and ADAM17 in HOKs

Following exposure to LPS, the protein levels of ADAM17 in the lysate of HOKs and TNF-α in the supernatant were assessed. In HOKs treated for 3 h and 12 h with 10 μg/mL *E. coli* and for 12 h with

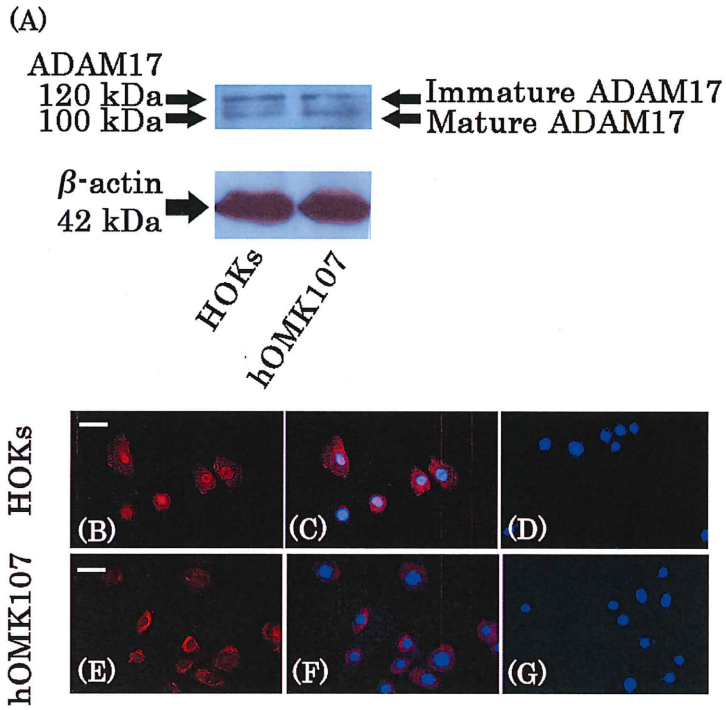

Fig. 2 Expression and subcellular distribution of ADAM17 in HOKs and hOMK107 cells. Protein expression of ADAM17 detected by immunoblotting. (A) Microscopy images of ADAM17 (red) (B and E), ADAM17 (red) counterstained with nuclear DAPI (blue) (C and F), and isotype control counterstained with nuclear DAPI (blue) (D and G). B–D and E–G show the images of HOKs and hOMK107, respectively. White bar, 30 μm.

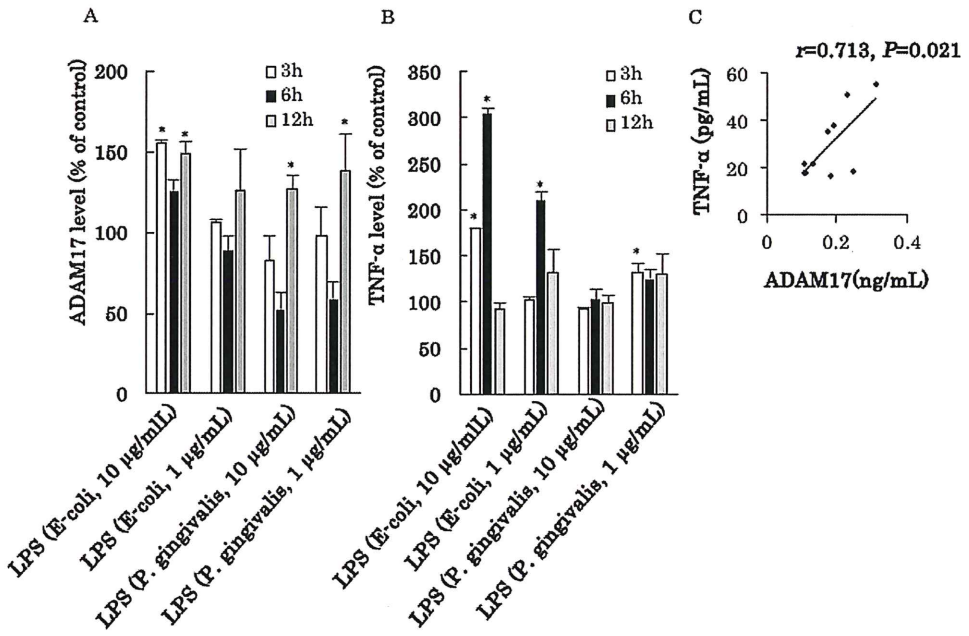

Fig. 3 The effect of LPS exposure on ADAM17 and TNF-α protein expression in HOKs. (**A**) Expression of ADAM17 in supernatant of HOK cultures treated with LPS. (**B**) Expression of TNF-α protein in HOKs treated with LPS. Cells were exposed to 10 μg/mL or 1 μg/mL of either *E. coli* or *P. gingivalis* LPS, and incubated for 3 (white bar), 6 (black bar), or 12 (grey bar) h. Bars and error bars represent the mean and SD, respectively, of three independent experiments. *$P < 0.05$ vs. untreated control cells (ANOVA with Dunnett's test). (**C**) Correlation between ADAM17 and TNF-α protein levels. r and P values were assessed by Spearman's ranking correlation analyses.

10 and 1 μg/mL *P. gingivalis* LPS, the mean levels of ADAM17 were significantly increased compared with untreated control cells (Fig. 3A). Furthermore, in HOKs treated for 3 h and 6 h with 10 μg/mL *E. coli* LPS, for 6 h with 1 μg/mL *E. coli* and for 3 h with 1 μg/mL *P. gingivalis* LPS, the mean levels of TNF-α were significantly increased compared with untreated control cells (Fig. 3B). Moreover, the protein expression of ADAM17 and TNF-α to *E. coli* LPS tended to react dose-dependently, while the protein expression levels of ADAM17 and TNF-α to 1 μg/mL *P. gingivalis* LPS increased higher compared with those to 10 μg/mL LPS.

Associations between the protein levels of ADAM17 in the lysate and TNF-α in the supernatant of HOKs treated with LPS under different conditions were assessed by Spearman's correlation analysis (Fig. 3C). Protein levels of TNF-α in the supernatant of cells were significantly correlated with those of ADAM17 in the cell lysate ($r = 0.713$).

Effect of TAPI-0 and GM1489 on TNF-α protein expression in HOKs exposed to LPS

TNF-α protein levels were determined in the supernatant of HOKs exposed to LPS (6 h, 10 μg/mL *E. coli* or *P. gingivalis*) with and without TAPI-0, GM1489, or both combined (Fig. 4). Compared with untreated control cells, TNF-α levels tended to increase in the presence of LPS. Furthermore, cells exposed to *E. coli* but not *P. gingivalis* LPS had significantly en-

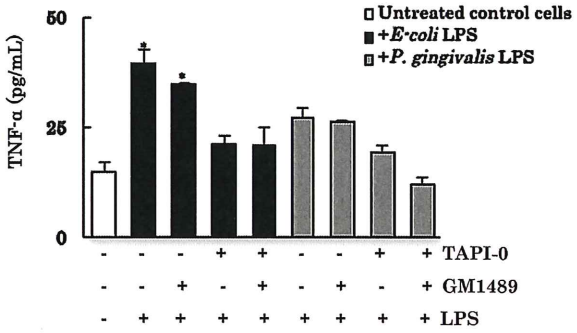

Fig. 4 Effect of TAPI-0 and GM1489 on TNF-α protein expression in HOKs exposed to LPS. Cells were incubated with 10 μg/mL *E. coli* or *P. gingivalis*, 100 nM TAPI-0, and/or 500 nM GM1489. White bar: TNF-α in untreated control cells; Black bar: TNF-α in cells treated with *E. coli* LPS; Grey bar: TNF-α in cells treated with *P. gingivalis* LPS. Bars and error bars represent the mean and SD, respectively, of three independent experiments. *$P < 0.05$ vs. untreated control cells.

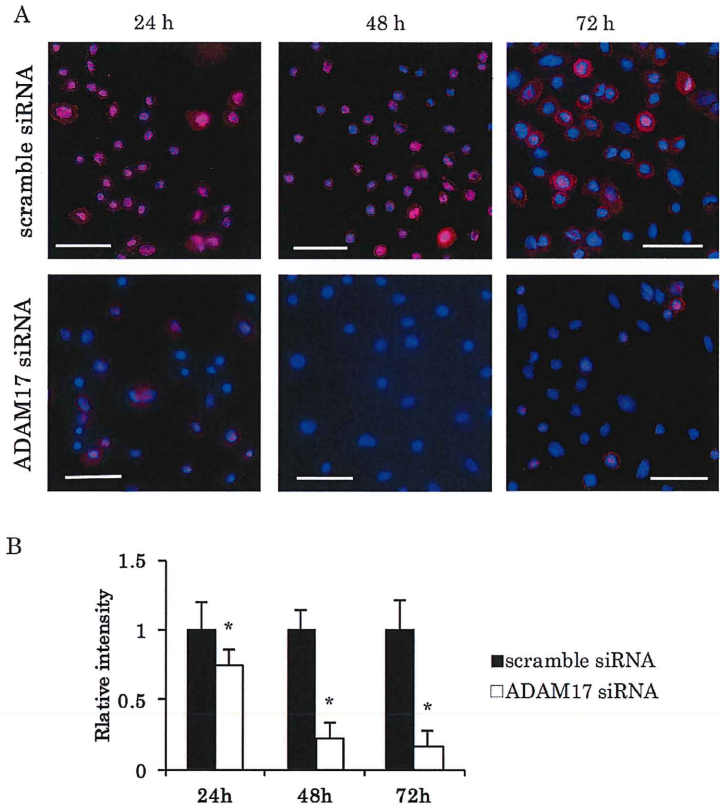

Fig. 5 Effects of siRNA targeting ADAM17 mRNA on HOKs. (**A**) HOKs stained for ADAM17 and counterstained with nuclear DAPI. White bar, 50 μm. (**B**) The relative fluorescence intensity for ADAM17. Cells were randomly selected after transfection and normalized to cell number. The mean fluorescence intensity for ADAM17 was measured using BZ-II imaging software (KEYENCE). Bars and error bars represent the mean and SD, respectively. Black bar, scramble siRNA; white bar, ADAM17 siRNA. $^*P < 0.05$ vs. scramble siRNA (*t*-test).

hanced TNF-α levels compared with untreated control cells. Treatment of cells with GM1489 or/and TAPI-0 in the presence of LPS tended to inhibit TNF-α levels. TAPI-0 inhibited LPS-induced TNF-α to a greater extent compared with the minimal inhibition observed by GM1489 alone.

Effect of siRNA targeting on ADAM17 expression in HOKs

Expression levels of ADAM17 in HOKs transfected with ADAM17 siRNA or scramble siRNA control were assessed by immunofluorescence at 24, 48, and 72 h post-transfection (Fig. 5). At 48 and 72 h post-transfection, ADAM17 was visibly down-regulated compared with control cells (Fig. 5A). Furthermore, the mean fluorescence intensity of ADAM17 at 24, 48, and 72 h post-transfection was significantly reduced to approximately 75%, 30%, and 20% of that in the control cells, respectively (Fig. 5B, $P < 0.05$, *t*-test).

Cell viability post-transfection was not significantly different between cells transfected with ADAM17 siRNA, cells transfected with scramble siRNA, or untreated control cells at 48 and 72 h. However, cell viability at 24 h after transfection with siRNA targeting ADAM17 and scramble siRNA was significantly lower compared with untreated control cells (Fig. 6).

The concentration of TNF-α in the supernatant of HOKs transfected with ADAM17 siRNA was measured at 72 h post-transfection to verify the role of ADAM17. When LPS was added to the ADAM17 siRNA transfected cells, the TNF-α level did not increase significantly compared to that in the cells without LPS. However, the TNF-α level was significantly higher in the control cells and the cells transfected with scramble siRNA compared with those without LPS (Fig. 7).

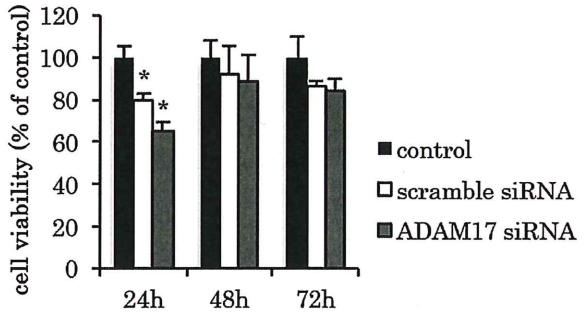

Fig. 6 Effects of ADAM17 siRNA on the viability of HOKs. Bars and error bars represent the mean and SD, respectively, of three independent experiments. Black bar, untreated control; white bar, scramble siRNA; gray bar, ADAM17 siRNA. *$P < 0.05$ vs. control (ANOVA with Dunnett's test).

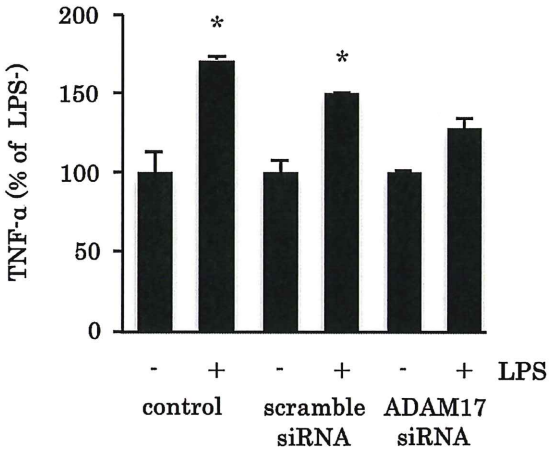

Fig. 7 The effect of ADAM17 siRNA on LPS-induced TNF-α expression from HOKs. TNF-α expression in the supernatant of HOKs exposed to 10 μg/mL *E. coli* LPS after transfection with ADAM17 siRNA was assessed by ELISA. Bars and error bars represent the mean and SD, respectively, of two independent experiments. *$P < 0.05$ vs. untreated control cells (*t*-test).

DISCUSSION

ADAM17 is widely expressed in various tissues including the brain, heart, kidney, and skeletal muscle (3), and is associated with many conditions including inflammatory diseases and malignancies (11). Our recent study indicated that several metalloendopeptidase genes, including *ADAM17*, are expressed in the buccal mucosal epithelium of patients with severe periodontitis (15). However, details on the protein expression of ADAM17 in human oral tissues and the relationship between ADAM17 and oral diseases have not been reported.

The present study demonstrates that ADAM17 is expressed in the oral epithelium of human gingival tissues and HOKs. In this study, however, only inflamed gingival tissues taken from sites of 'bleeding on probing' were used. Therefore, differences in the presence of ADAM17 in the oral epithelium between inflamed and non-inflamed gingival tissues remain unclear. However, ADAM17 is assumed to be expressed at lower level in the epithelium of the non-inflamed gingival tissues compared to inflamed gingival tissues, since a previous study has already reported that a low level of ADAM17 is selectively expressed in epithelial basal layer and supra-basal layer cells and in some lamina propria fibroblasts of normal oral mucosa tissues compared to ADAM17 expression in the cancer cells of head and neck cancer tissues at higher level (10). Although further studies are needed to demonstrate that ADAM17 is related to oral diseases such as periodontitis, ADAM17 expression in gingival tissues is assumed to change according to periodontal health status.

Previous studies suggest that the majority of the active form of ADAM17 is localized in the perinuclear region, with a small amount present in the plasma membrane (11, 20). The immunofluorescence results in this study also indicate that ADAM17 is predominantly expressed in the perinuclear region in oral keratinocytes in addition to the cytoplasm and nucleus. The prodomain of ADAM17 is thought to be cleaved by furin, a pro-protein convertase, in the trans-Golgi network (20). Therefore, ADAM17 in oral keratinocytes may be converted from an immature to mature form in the trans-Golgi network.

Another study reported that LPS treatment *in vitro* and *in vivo* increased ADAM17 mRNA expression in cultured lung endothelial cells and in the lungs of mice, respectively, and that the upregulation was associated with an increase in ADAM17 activity and cleavage of ADAM17 substrates within a similar time frame (6). In the present study, stimulation by LPS originating from *E. coli* and *P. gingivalis* was used to induce TNF-α and ADAM17 release from cells. LPS stimulation enhanced the level of ADAM17 and TNF-α secreted from oral keratinocytes, although differences in the level of enhancement existed with respect to the origin, concentration and time of LPS exposure to the cells. Previous studies report that the signaling pathway of *P. gingivalis* LPS differs from that of *E. coli* LPS in cells (22), which leads to differential immune responses with respect to mRNA expression levels and the time course of each cytokine (5). Thus, differences in TNF-α levels released from cells following *E.*

coli and *P. gingivalis* LPS stimulation may relate to differences in the signaling pathways. In this study, *E. coli* LPS was a more useful inducer of TNF-α to verify the influence of ADAM17 on the release of TNF-α in oral keratinocytes, since the aim of this study was not to investigate the signaling pathway of LPS. Conversely, *P. gingivalis* LPS was more suitable to verify the relationship between TNF-α and periodontitis. Furthermore, significant correlation existed between the levels of TNF-α and ADAM17, which were simultaneously induced by a variety of LPS stimulation. These findings suggest that the level of ADAM17 induced by LPS treatment in oral keratinocytes might directly influence that of TNF-α secreted from the cells in the short period of time.

TNF-α is a key pro-inflammatory cytokine within destructive periodontal disease. Elevated levels of TNF-α from various cells in gingival tissue are associated with the destruction of periodontal tissues, including bone resorption (12). Therefore, TNF-α in the early inflammatory stage of periodontitis is regarded as a therapeutic target, and the inhibition of the enzyme involved in TNF-α cleavage is an important strategy for the regulation of inflammation linked to TNF-α. Although ADAM17 has emerged as a candidate enzyme responsible for the production of TNF-α, other proteases, including MMP-7, have also been implicated in releasing TNF-α (13, 19). In the present study, we considered whether TNF-α induced by LPS stimulation was influenced not only by ADAM17, but also other enzymes, such as MMPs. To investigate this, an ADAM17 inhibitor (TAPI-0) (9) and a potent broad-range MMP inhibitor (GM1489) (14) were used to investigate the inhibitory effects of TNF-α expression induced by LPS stimulation. TAPI-0 was found to inhibit not only ADAM17 but also some MMPs, whilst GM1489 inhibited many MMPs but not ADAM17. Therefore, we considered that the difference in the response to TAPI-0 versus GM1489 was influenced by the inhibition of ADAM17. The present study indicates that TAPI-0 inhibited LPS-induced expression of TNF-α to a greater extent compared with GM1489. This suggests that LPS-induced TNF-α is regulated by the action of ADAM17. Additionally, this study indicates that ADAM17 siRNA can effectively down-regulate ADAM17 expression in oral keratinocytes and that LPS treatment of these cells does not induce TNF-α. Thus, ADAM17 appears to be a key enzyme for the production of TNF-α in oral keratinocytes.

In conclusion, the present study has demonstrated for the first time that ADAM17 is expressed in the epithelium of gingival tissues and that it acts as an enzyme regulating the generation of TNF-α in oral keratinocytes. This may lead to a novel approach for the prevention and treatment of oral diseases such as periodontitis.

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