RESEARCH

Expression of deubiquitinases in human gingiva and cultured human gingival fibroblasts

Yong-Wei Fu[*] and Hong-Zhi Xu

Abstract

Background: Although deubiquitinating enzymes (DUBs) such as CYLD, A20 and OTULIN are expressed in multiple tissues and thought to be linked with inflammatory diseases, their expression in periodontal tissues remains to be determined. This research was designed to assess the expression of CYLD, A20 and OTULIN in human gingiva, and to evaluate the regulation of these DUBs in human gingival fibroblasts (HGFs) upon different stimuli.

Methods: Immunohistochemistry assay was conducted to determine the expression of CYLD, A20 and OTULIN in human gingiva. Immunofluorescence assay was employed to observe the protein expression of CYLD, A20 and OTULIN in HGFs. RT-PCR and western blots were carried out to assess gene and protein expression changes of these DUBs in HGFs upon LPS or TNF-α.

Results: CYLD, A20 and OTULIN were found to be expressed in human gingiva and HGFs. The expression of CYLD, A20 and OTULIN was lower in the inflamed gingival tissue samples compared with the healthy gingival tissue samples. Further, the expression of CYLD, A20 and OTULIN in HGFs exhibited distinct regulation by different stimuli. TNF-α treatment markedly increased NF-κB activation in HGFs

Conclusions: Our findings suggest that CYLD, A20 and OTULIN might play a role in the progression of periodontitis.

Keywords: CYLD, A20, OTULIN, Gingiva, Fibroblasts

Background

Ubiquitin, a protein containing 76 amino acids, has an essential role in variety of biological processes [1–3]. Ubiquitination is a critical post-translational protein modification, performed by specific ubiquitin enzymes [4, 5]. The ubiquitin enzymes attach ubiquitin to target proteins and modulate the function of the target proteins. Attached ubiquitins can be removed by deubiquitinating enzymes (DUBs). Several DUBs including CYLD, A20 and OTULIN are reported to inhibit NF-κB activation and play an important role in modulating immunity and inflammation [6–8]. Furthermore, previous studies

have shown that CYLD, A20 and OTULIN are all related to inflammatory diseases [9–12].

Periodontitis is an inflammatory disease of periodontal tissues which is caused by plaque biofilms [13, 14]. In addition to being an important cause of tooth loss in adults, periodontitis is highly linked with multiple systemic diseases, including Alzheimer's disease, cardiovascular disease, diabetes, rheumatoid arthritis and oral cancer [14–17]. Recent work suggests that dysregulation of immune and inflammatory responses in the periodontium may be involved in the progression of periodontitis [18, 19]. Briefly, periodontal pathogenic bacteria and their toxic substances interact with host immune cells, result in immune and inflammatory responses in periodontal tissues, trigger the excessive release of inflammatory cytokines, and eventually cause periodontal tissue destruction [20]. Recent work demonstrates that

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regulating the immune responses may act as an adjunct to traditional periodontal therapy [21, 22].

NF-κB is an important regulator of immune and inflammatory responses [7, 23]. Upregulated NF-κB activity is reported in many inflammatory conditions such as inflammatory bowel disease, rheumatoid arthritis, and periodontitis [24, 25]. Evidence increasingly suggests that NF-κB activation may be involved in the progression of periodontitis [24, 26]. Periodontal pathogens may promote the induction of proinflammatory cytokines in periodontal tissues by upregulating NF-κB activation [20]. Considering the repressing effect of CYLD, A20 and OTULIN on NF-κB activation, it is reasonable to postulate that these DUBs may restrict periodontal inflammatory responses.

Although CYLD, A20 and OTULIN are expressed in multiple tissues, their expression in periodontal tissues remains to be elucidated. The objective of this research is to assess the expression of CYLD, A20 and OTULIN in human gingiva and to evaluate the regulation of these DUBs in HGFs upon different stimuli.

Methods

Antibodies and reagents

Rabbit anti-CYLD (catalog #ab137524), rabbit anti-A20 (catalog #ab92324) and rabbit anti-OTULIN (catalog #ab151117) were purchased from Abcam (Cambridge, UK). Rabbit anti-p65 (catalog #8242), Rabbit anti-phospho-p65 (catalog #3033), mouse anti-IκBα (catalog #4814), mouse anti-phospho-IκBα (catalog #9246) were purchased from CST (Danvers, MA, USA). Normal rabbit IgG (catalog #A7016) were purchased from Beyotime Biotechnology (Shanghai, China). Alexa Fluor 568 goat anti-rabbit IgG (catalog #A11036) was supplied by Thermo Fisher Scientific (Rockford, IL, USA). Tubulin rabbit polyclonal antibody (catalog #10094-1-AP), GAPDH rabbit polyclonal antibody (catalog #10494-1-AP), horseradish peroxidase-conjugated goat anti-mouse IgG (catalog #SA00001-1) and horseradish peroxidase-conjugated goat anti-rabbit IgG (catalog #SA00001-2) were supplied by Proteintech Group (Wuhan, China). Minimum essential medium alpha was supplied by Thermo Fisher Scientific (Suzhou, China). Penicillin–streptomycin solution (catalog #SV30010) was supplied by HyClone (Austria). Fetal bovine serum (catalog #VS500T) was supplied by Ausbian (Australia). Immunohistochemistry application solutions kit (catalog #13079) was supplied by Cell Signaling Technologies (Danvers, MA, USA). DAPI (catalog #R37606) and Pro-Long Diamond Antifade Mountant (catalog #P36965) were supplied by Molecular Probes (Eugene, OR, USA). TNF-α (catalog #01375) was supplied by R&D Systems (Minneapolis, MN, USA). LPS from *Porphyromonas gingivalis* (catalog #tlrl-pglps) was supplied by InvivoGen (San Diego, USA). RNA extraction kit (catalog #R6834-01) was from OMEGA Bio-Tek (Norcross, GA, USA). PrimeScript RT Master Mix kit (catalog #RR036A) and SYBR Premix Ex Taq II kit (catalog #RR820A) were from Takara Bio Inc. (Otsu, Japan). BCA Protein Assay Kit (catalog #23227) was from Pierce Biotechnology (Rockford, IL, USA).

Gingival samples

Healthy gingival samples were acquired from 12 individuals (5 male and 7 female, average age of 28.7 years) undergoing crown-lengthening surgery. Inflamed gingival samples were obtained from 9 individuals (6 male and 3 female, average age of 35.3 years) with chronic periodontitis during periodontal flap surgery. The inclusion and exclusion criteria were the same as those used in a previous study from our group [27]. All individuals were selected from the First People's Hospital of Lianyungang between March 2018 and August 2019. Informed consent forms were assigned by all individuals before they were enrolled in the research. This study was approved by The Ethics Committee of Nanjing Medical University (2018389). All experiments on human subjects were conducted in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Immunohistochemistry assay

Gingival samples were fixed in 4% paraformaldehyde for 24 h at 4 °C. Then these samples were processed to paraffin blocks and sectioned at 5 μm. Immunohistochemical study was undertaken as previously described using a commercial immunohistochemistry kit [27]. Anti-CYLD (1:400), anti-A20 (1:400) and anti-OTULIN (1:400,) antibodies were applied in this study. Nonimmunized IgG (1:400, Beyotime Biotechnology) was used as negative controls in the present study. Slices were photographed with a Leica DM4000 B microscope.

Cell culture

HGFs were isolated from human gingival tissues and cultured with an explant culture method. Briefly, gingival tissues were acquired from three healthy individuals (2 females and 1 male, aged 11–14 years) experiencing first premolar extractions for orthodontic therapy. The extracted teeth were placed in phosphate buffered saline (PBS) and immediately transferred to the laboratory. Then the gingival tissues attached to the cervical region of the extracted teeth were removed and cut into small pieces in the biosafety cabinet. Afterwards, the tissue pieces were transferred to 25 cm² culture flasks and cultured in minimum essential medium alpha supplemented

with 15% fetal bovine serum. HGFs at passages 3 to 6 were used in this study.

Immunofluorescence staining

For Immunofluorescence Staining, HGFs were seed in 24-well culture plates with a coverslip at the bottom in a density of 2.5×10^4 cells per well. After 24 h, HGFs were fixed with 4% paraformaldehyde in PBS for 15 min, permeabilized with 0.2% Triton X-100 in PBS for 5 min, blocked with 1% BSA in PBS for 1 h at room temperature (RT). Then HGFs were incubated with primary antibodies against CYLD (1:200), A20 (1:200) and OTULIN (1:200) overnight at 4 °C. Afterwards, HGFs were stained with Alexa Fluor 568-conjugated secondary antibody (4 μg/mL) for 50 min and then with DAPI for 6 min at RT. Each of the above-mentioned steps was followed by three 5-min washes in PBS. Finally, coverslips were mounted using ProLong Diamond Antifade Mountant and data were obtained by using a Leica DM4000 B microscope.

Cell stimulation

HGFs were seed in 6-well culture plates and cultured for 24 h. After washing twice with PBS, HGFs were starved for 12 h in α-MEM without foetal bovine serum. Then HGFs were stimulated with 1 μg/mL LPS or 10 ng/ml TNF-α as indicated.

RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from treated HGFs with an RNA extraction kit per the supplier's protocol. cDNA synthesis was undertaken using a PrimeScript RT Master Mix kit. Then RT-PCR was carried out using a SYBR Premix Ex Taq II kit on a 7300 Real Time PCR System. Each sample was run in triplicate. Results were normalized to GAPDH. Primers were: CYLD, 5′-ACGCCACAATCT TCATCACACT-3′ (forward:) and 5′-AGGTCGTGG TCAAGGTTTCACT-3′ (reverse);; *A20*, 5′-ATGCAC CGATACACACTGGA-3′ (forward) and 5′-GGATGA TCTCCCGAAACTGA-3′ (reverse); *OTULIN*, 5′-AAA GAGGGGCATCAGAACCG-3′ (forward) and 5′-GGC CCTCAGTGCACAGTAAT-3′ (reverse); and GAPDH, 5′-TGCACCACCAACTGCTTAGC-3′ (forward) and 5′-GGCATGGA-CTGTGGTCATGAG-3′ (reverse).

Protein extraction and western blotting

For western blotting, pre-treated HGFs were lysed using RIPA lysis buffer containing protease inhibitors. The protein concentrations were determined with a BCA Protein Assay Kit. Then, the protein extracts were subjected to western blotting with antibodies to CYLD (1:1000), A20 (1:1000), OTULIN (1:400), p65 (1:1000), phospho-p65 (1:1500), IκBα (1:1000), phospho-IκBα (1:1000), Tubulin (1:1000) and GAPDH (1:10,000). Horseradish peroxidase-conjugated goat anti-mouse IgG (1:8000) and Horseradish peroxidase-conjugated goat anti-rabbit IgG (1:8000) were applied. Densitometry quantification of the protein bands was processed with ImageJ software.

Statistical analysis

Data are expressed as the mean ± SD. ANOVA followed by Dunnett's multiple comparison test was used to determine the significance of differences. GraphPad Prism 5 was used to perform statistical analyses. Differences were considered to be significant when $P < 0.05$.

Results

Expression of deubiquitinases in human gingival tissues

To determine the expression of CYLD, A20 and OTULIN in human gingiva, we performed immunohistochemistry staining on healthy and inflamed gingival tissue samples. Positive staining cells for CYLD, A20and OTULIN were observed both in the epithelium and in the connective tissue (Fig. 1). All of the above deubiquitinases were mainly expressed in epithelial cells. Further, the expression of CYLD, A20 and OTULIN was lower in the inflamed gingival tissue samples compared with the healthy gingival tissue samples (Fig. 1).
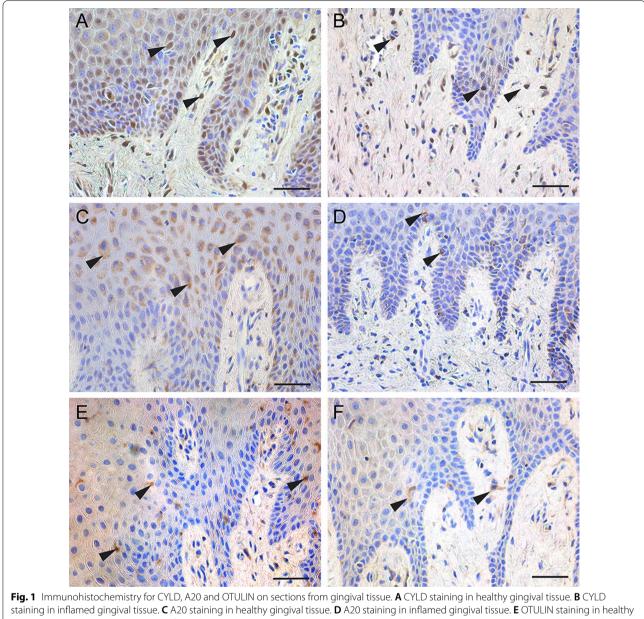
Expression of deubiquitinases in human gingival fibroblasts

Immunofluorescence was performed to assess the expression of deubiquitinases in HGFs. As expected, HGFs displayed positive staining for CYLD, A20 and OTULIN. (Fig. 2).

Regulation of deubiquitinases by different stimuli

To examine the changes in the expression levels of deubiquitinases in LPS- or TNF-α-treated HGFs, RT-PCR and western blotting were performed. Results demonstrated that the expression of CYLD, A20 and OTULIN in HGFs exhibits distinct regulation upon LPS or TNF-α (Figs. 3, 4).

The RT-PCR results demonstrated that the CYLD mRNA level had no obvious change after treatment with LPS or TNF-α (Fig. 3A, D). Intriguingly, the mRNA expression of A20 was decreased 1 h after treatment with LPS, and returned to the baseline level after 5 h (Fig. 3B). As expected, the mRNA expression of A20 was rapidly upregulated in response to TNF-α (Fig. 3E). In addition, LPS led to an increase in OTULIN mRNA expression after 3 h (Fig. 3C). In contrast, TNF-α resulted in a decrease in the mRNA expression of OTULIN after 1 h (Fig. 3F).

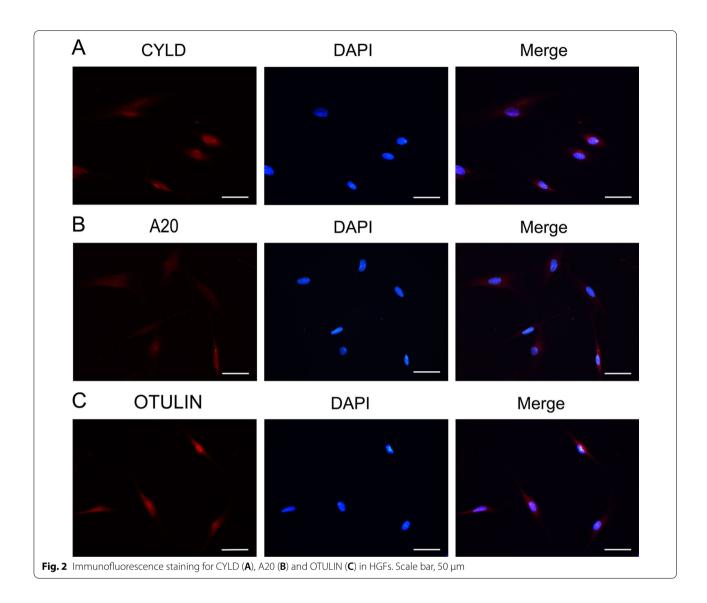**Fig. 1** Immunohistochemistry for CYLD, A20 and OTULIN on sections from gingival tissue. **A** CYLD staining in healthy gingival tissue. **B** CYLD staining in inflamed gingival tissue. **C** A20 staining in healthy gingival tissue. **D** A20 staining in inflamed gingival tissue. **E** OTULIN staining in healthy gingival tissue. **F** OTULIN staining in inflamed gingival tissue. Arrows show positive staining for CYLD (**A**, **B**), A20 (**C**, **D**) and OTULIN (**E**, **F**). Scale bar, 50 μm

At the protein level, CYLD expression had no significant change upon LPS or TNF-α treatment (Fig. 4A, B). However, after 2 h of treatment with LPS, the protein level of CYLD was slightly higher than the baseline. In contrast, after 1 h of treatment with TNF-α, the CYLD protein level was slightly lower than the baseline. In addition, the protein expression of A20 markedly increased after treatment with LPS or TNF-α (Fig. 4A, B). Furthermore, LPS also led to the increase of OTULIN protein expression (Fig. 4A). However, TNF-α treatment did not affect the protein expression of OTULIN (Fig. 4B).

NF-κB activation in HGFs upon different stimuli
To evaluate NF-κB activation in HGFs upon LPS or TNF-α, western blotting was carried out. We found

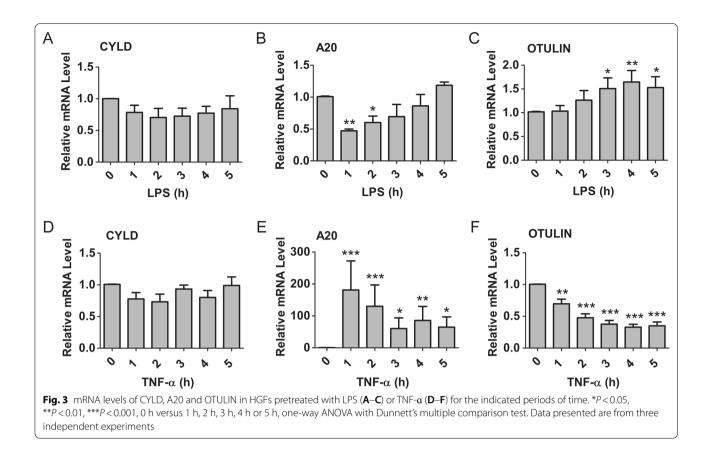**Fig. 2** Immunofluorescence staining for CYLD (**A**), A20 (**B**) and OTULIN (**C**) in HGFs. Scale bar, 50 μm

that LPS treatment had no obvious effect on the activation of NF-κB in HGFs (Fig. 5A). Expectedly, TNF-α treatment markedly increased NF-κB activation in HGFs (Fig. 5B).

Discussion

In the presented study, we examined the expression of different DUBs in human gingiva and HGFs. We found that both biopsies of human gingiva and HGFs show the expression of CYLD, A20 and OTULIN. In addition, the inflamed gingival tissue samples display reduced expression of CYLD, A20 and OTULIN when compared with the healthy gingival tissue samples. Furthermore, the expression of CYLD, A20 and OTULIN in HGFs exhibits distinct regulation by different

stimuli. We also found that TNF-α treatment markedly increased NF-κB activation in HGFs. It is known that DUBs such as CYLD, A20 and OTULIN can inhibit inflammatory responses by repressing NF-κB and other signaling pathways [9, 10, 28, 29]. Our findings thus imply that CYLD, A20 and OTULIN may play a part in periodontal inflammatory responses.

Previous studies demonstrated that CYLD, A20 and OTULIN are all critical players in suppressing inflammation and immunity [6, 8, 29]. The deficiency of these DUBs may lead to constitutive NF-κB responses, enhance NF-κB-dependent gene expression, and result in excessive production of proinflammatory cytokines [4, 7, 8]. In our present study, we characterized the expression of CYLD, A20 and OTULIN in human gingiva and HGFs.

Fig. 3 mRNA levels of CYLD, A20 and OTULIN in HGFs pretreated with LPS (**A**–**C**) or TNF-α (**D**–**F**) for the indicated periods of time. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, 0 h versus 1 h, 2 h, 3 h, 4 h or 5 h, one-way ANOVA with Dunnett's multiple comparison test. Data presented are from three independent experiments
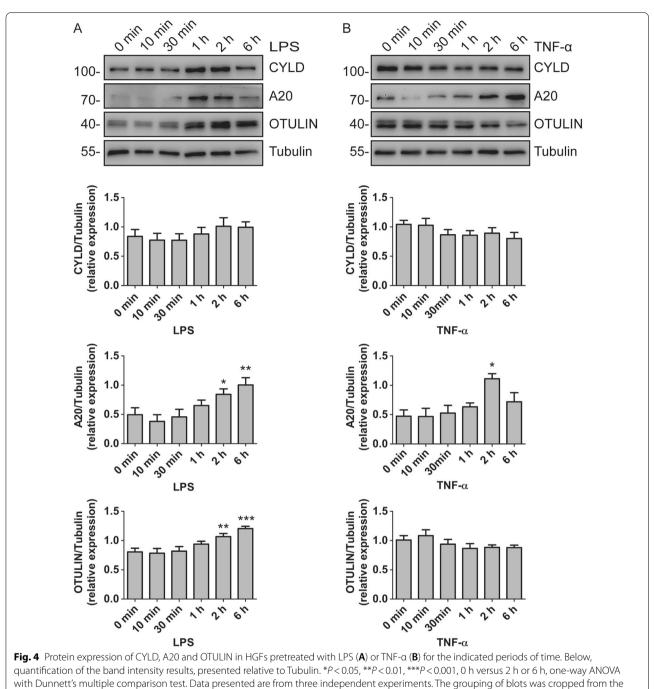
In line with our findings, a previous study also confirmed A20 expression in human gingival tissue [30]. Given the role of these DUBs in inhibiting inflammation, we postulate that these DUBs may be involved in the development of periodontitis.

It is known that CYLD expression can be upregulated by many stimuli. In the present study, we found that both LPS and TNF-α did not increase the mRNA level of CYLD. Similarly, western blotting data also showed that the protein level of CYLD had no significant change in the presence of LPS or TNF-α. However, after 2 h of treatment with LPS, the protein level of CYLD was slightly higher than the baseline. In contrast, after 1 h of treatment with TNF-α, the CYLD protein level was slightly lower than the baseline. Several early reports indicated that CYLD expression was upregulated by LPS or TNF-α treatment [31–33]. The potential mechanisms for this discrepancy remain unknown, but probably due to cell-type-specific differences.

A20, a key regulator of inflammation, is expressed at a low level in most cell types and can be induced by various stimuli including TNF-α, LPS, and IL-1 [8]. In this study, the mRNA level of A20 was markedly upregulated by TNF-α. In contrast, LPS treatment led

to a decrease in A20 mRNA expression. As expected, both LPS and TNF-α led to the increase of A20 protein expression. We found a lack of concordance between the protein and mRNA levels of A20 in HGFs upon LPS treatment. This result was unexpected but interesting. Many prior works have demonstrated that the expression levels of mRNA and protein are often poorly correlated [34–36]. This discrepancy may be attributed to other types of regulation, such as post-transcriptional processing and protein degradation [34, 35]. In addition, several recent studies indicated that A20 may act as a critical regulator in the pathogenesis of periodontitis [30, 37–39].

OTULIN was identified as the deubiquitinase that specifically hydrolyzes linear polyubiquitin chains and suppresses NF-κB activation [40]. Recent work suggested that OTULIN can limit inflammation by deubiquitinating LUBAC [9]. However, the regulation of OTULIN is yet to be determined. A recent work showed that porcine reproductive and respiratory syndrome virus infection upregulates the expression of OTULIN [41]. In our current study, LPS resulted in the increase of the mRNA and protein expression of OTULIN. In contrast, the OTULIN mRNA level was decreased upon TNF-α stimulation. Unexpectedly,
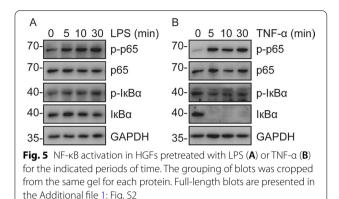
Fig. 4 Protein expression of CYLD, A20 and OTULIN in HGFs pretreated with LPS (**A**) or TNF-α (**B**) for the indicated periods of time. Below, quantification of the band intensity results, presented relative to Tubulin. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, 0 h versus 2 h or 6 h, one-way ANOVA with Dunnett's multiple comparison test. Data presented are from three independent experiments. The grouping of blots was cropped from the same gel for each protein. Full-length blots are presented in the Additional file 1: Fig. S1

TNF-α treatment did not change the protein expression of OTULIN. Further studies need to be carried out to investigate the precise mechanism of OTULIN regulation.

Conclusions

In summary, we show that the DUBs CYLD, A20 and OTULIN are expressed in human gingival tissues and are distinctly regulated by different stimuli. Given the role of these DUBs in limiting inflammation, our findings suggest that CYLD, A20 and OTULIN might be involved in the pathogenesis of periodontitis and

Fig. 5 NF-κB activation in HGFs pretreated with LPS (**A**) or TNF-α (**B**) for the indicated periods of time. The grouping of blots was cropped from the same gel for each protein. Full-length blots are presented in the Additional file 1: Fig. S2

might act as potential therapeutic targets to treat periodontitis. Therefore, the exact effects of these DUBs on periodontal inflammatory responses require further investigation.

Abbreviations
DUBs: Deubiquitinating enzymes; HGFs: Human gingival fibroblasts; PBS: Phosphate buffered saline; RT-PCR: Reverse transcription polymerase chain reaction.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12903-021-01655-4.

Additional File 1. Full-length blots shown in Figs. 4 and 5.

Authors' contributions
Y-WF is the major person to complete this study. H-ZX is the major person to write the paper. Both authors read and approved the final manuscript.

Availability of data and materials
The datasets used in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by The Ethics Committee of Nanjing Medical University (2018389). Informed consent forms were assigned by all individuals before they were enrolled in the research.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Ciechanover A. The ubiquitin-proteasome proteolytic pathway. Cell. 1994;79(1):13–21.
2. Goldstein G, Scheid M, Hammerling U, Schlesinger DH, Niall HD, Boyse EA. Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proc Natl Acad Sci USA. 1975;72(1):11–5.
3. Swatek KN, Komander D. Ubiquitin modifications. Cell Res. 2016;26(4):399–422.
4. Iwai K, Fujita H, Sasaki Y. Linear ubiquitin chains: NF-kappaB signalling, cell death and beyond. Nat Rev Mol Cell Biol. 2014;15(8):503–8.
5. Witt A, Vucic D. Diverse ubiquitin linkages regulate RIP kinases-mediated inflammatory and cell death signaling. Cell Death Differ. 2017;24(7):1160–71.
6. Aksentijevich I, Zhou Q. NF-kappab pathway in autoinflammatory diseases: dysregulation of protein modifications by ubiquitin defines a new category of autoinflammatory diseases. Front Immunol. 2017;8:399.
7. Zhang Q, Lenardo MJ, Baltimore D. 30 Years of NF-kappaB: a blossoming of relevance to human pathobiology. Cell. 2017;168(1–2):37–57.
8. Lork M, Verhelst K, Beyaert R. CYLD, A20 and OTULIN deubiquitinases in NF-kappaB signaling and cell death: so similar, yet so different. Cell Death Differ. 2017;24(7):1172–83.
9. Heger K, Wickliffe KE, Ndoja A, Zhang J, Murthy A, Dugger DL, Maltzman A, de Sousa EMF, Hung J, Zeng Y, et al. OTULIN limits cell death and inflammation by deubiquitinating LUBAC. Nature. 2018;559(7712):120–4.
10. Ji YX, Huang Z, Yang X, Wang X, Zhao LP, Wang PX, Zhang XJ, Alves-Bezerra M, Cai L, Zhang P, et al. The deubiquitinating enzyme cylindromatosis mitigates nonalcoholic steatohepatitis. Nat Med. 2018;24(2):213–23.
11. Polykratis A, Martens A. A20 prevents inflammasome-dependent arthritis by inhibiting macrophage necroptosis through its ZnF7 ubiquitin-binding domain. Nat Cell Biol. 2019;21(6):731–42.
12. Priem D, van Loo G, Bertrand MJM. A20 and cell death-driven inflammation. Trends Immunol. 2020;41(5):421–35.
13. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, et al. Periodontitis: consensus report of workgroup 2 of the 2017 World workshop on the classification of periodontal and peri-implant diseases and conditions. J Periodontol. 2018;89(Suppl 1):S173-s182.
14. Fiorillo L, Cervino G, Laino L, D'Amico C, Mauceri R, Tozum TF, Gaeta M, Cicciù M. *Porphyromonas gingivalis*, periodontal and systemic implications: a systematic review. Dent J. 2019;7(4):114.
15. Hasturk H, Kantarci A. Activation and resolution of periodontal inflammation and its systemic impact. Periodontol 2000. 2015;69(1):255–73.
16. Cervino G, Terranova A, Briguglio F, De Stefano R, Fama F, D'Amico C, Amoroso G, Marino S, Gorassini F, Mastroieni R, et al. Diabetes: oral health related quality of life and oral alterations. Biomed Res Int. 2019;2019:5907195.
17. Fiorillo L. Oral Health: the first step to well-being. Medicina (Kaunas). 2019;55(10):676.
18. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. Periodontol 2000. 2015;69(1):7–17.
19. Ebersole JL, Graves CL, Gonzalez OA, Dawson D III, Morford LA, Huja PE, Hartsfield JK Jr, Huja SS, Pandruvada S, Wallet SM. Aging, inflammation, immunity and periodontal disease. Periodontol 2000. 2016;72(1):54–75.
20. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000. 2014;64(1):57–80.
21. Van Dyke TE. Commentary: periodontitis is characterized by an immuno-inflammatory host-mediated destruction of bone and connective tissues that support the teeth. J Periodontol. 2014;85(4):509–11.
22. Bartold PM, Van Dyke TE. Host modulation: controlling the inflammation to control the infection. Periodontol 2000. 2017;75(1):317–29.
23. Taniguchi K, Karin M. NF-kappaB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol. 2018;18(5):309–24.
24. Ambili R, Janam P, Saneesh Babu PS, Prasad M, Vinod D, Anil Kumar PR, Kumary TV, Asha Nair S. Differential expression of transcription factors

NF-kappaB and STAT3 in periodontal ligament fibroblasts and gingiva of healthy and diseased individuals. Arch Oral Biol. 2017;82:19–26.

25. Garcia-Carbonell R, Wong J, Kim JY, Close LA, Boland BS, Wong TL, Harris PA, Ho SB, Das S, Ernst PB, et al. Elevated A20 promotes TNF-induced and RIPK1-dependent intestinal epithelial cell death. Proc Natl Acad Sci USA. 2018;115(39):E9192–200.

26. Golz L, Memmert S, Rath-Deschner B, Jager A, Appel T, Baumgarten G, Gotz W, Frede S. Hypoxia and *P. gingivalis* synergistically induce HIF-1 and NF-kappaB activation in PDL cells and periodontal diseases. Mediat Inflamm. 2015;2015:438085.

27. Fu YW, Li L, Wang XQ, Zhou Y, Zhu LF, Mei YM, Xu Y. The inhibitory effect of the deubiquitinase cylindromatosis (CYLD) on inflammatory responses in human gingival fibroblasts. Oral Dis. 2020. https://doi.org/10.1111/odi.13672.

28. Malynn BA, Ma A. A20: a multifunctional tool for regulating immunity and preventing disease. Cell Immunol. 2019;340:103914.

29. Wertz IE, Newton K, Seshasayee D, Kusam S, Lam C, Zhang J, Popovych N, Helgason E, Schoeffler A, Jeet S, et al. Phosphorylation and linear ubiquitin direct A20 inhibition of inflammation. Nature. 2015;528(7582):370–5.

30. Crump KE, Oakley JC, Xia-Juan X, Madu TC, Devaki S, Mooney EC, Sahingur SE. Interplay of toll-like receptor 9, myeloid cells, and deubiquitinase A20 in periodontal inflammation. Infect Immun. 2017;85(1):e00814-e816.

31. Jono H, Lim JH, Chen LF, Xu H, Trompouki E, Pan ZK, Mosialos G, Li JD. NF-kappaB is essential for induction of CYLD, the negative regulator of NF-kappaB: evidence for a novel inducible autoregulatory feedback pathway. J Biol Chem. 2004;279(35):36171–4.

32. Fukuda M, Fukuda F, Horiuchi Y, Oku Y, Suzuki S, Kusama K, Sakashita H. Expression of CYLD, NF-kappaB and NF-kappaB-related factors in salivary gland tumors. In Vivo (Athens, Greece). 2006;20(4):467–72.

33. Bikker R, Christmann M, Preuß K, Welz B, Friesenhagen J, Dittrich-Breiholz O, Huber R, Brand K. TNF phase III signalling in tolerant cells is tightly controlled by A20 and CYLD. Cell Signal. 2017;37:123–35.

34. Chen G, Gharib TG, Huang CC, Taylor JM, Misek DE, Kardia SL, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM, et al. Discordant protein and mRNA expression in lung adenocarcinomas. Mol Cell Proteomics. 2002;1(4):304–13.

35. de Sousa AR, Penalva LO, Marcotte EM, Vogel C. Global signatures of protein and mRNA expression levels. Mol BioSyst. 2009;5(12):1512–26.

36. Griffin TJ, Gygi SP, Ideker T, Rist B, Eng J, Hood L, Aebersold R. Complementary profiling of gene expression at the transcriptome and proteome levels in *Saccharomyces cerevisiae*. Mol Cell Proteomics. 2002;1(4):323–33.

37. Hong JY, Bae WJ, Yi JK, Kim GT, Kim EC. Anti-inflammatory and anti-osteoclastogenic effects of zinc finger protein A20 overexpression in human periodontal ligament cells. J Periodontal Res. 2016;51(4):529–39.

38. Li Y, Mooney EC, Holden SE, Xia XJ, Cohen DJ, Walsh SW, Ma A, Sahingur SE. A20 Orchestrates inflammatory response in the oral mucosa through restraining NF-kappaB activity. J Immunol. 2019;202(7):2044–56.

39. Mooney EC, Sahingur SE. The ubiquitin system and A20: implications in health and disease. J Dent Res. 2021;100(1):10–20.

40. Keusekotten K, Elliott PR, Glockner L, Fiil BK, Damgaard RB, Kulathu Y, Wauer T, Hospenthal MK, Gyrd-Hansen M, Krappmann D, et al. OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. Cell. 2013;153(6):1312–26.

41. Su Y, Shi P, Zhang L, Lu D, Zhao C, Li R, Zhang L, Huang J. The superimposed deubiquitination effect of OTULIN and porcine reproductive and respiratory syndrome virus (PRRSV) Nsp11 promotes multiplication of PRRSV. J Virol. 2018;92(9):e00175-e218.

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