International Immunopharmacology 77 (2019) 105956

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

LOX-1 is involved in TLR2 induced RANKL regulation in peri-implantitis

Qian Zhang[a,*], Jie Liu[a], Lei Ma[a], Na Bai[a], Huirong Xu[b]

[a] Department of Prosthodontics, The Affiliated Hospital of Qingdao University, Qingdao, Shandong Province, China
[b] Department of Pathology, ZiBo Central Hospital, ZiBo, Shandong Province, China

ARTICLE INFO

Keywords:
peri-implantitis
Porphyromonas gingivalis
RANKL
TLR2
LOX-1

ABSTRACT

Purpose: To explore whether receptor activator of nuclear factor kappa-B ligand (RANKL) is involved in the nosogenesis of peri-implantitis and to reveal the regulatory mechanism in *Porphyromonas gingivalis* induced RANKL production.

Methods: Therefore, we collected peri-implant crevicular fluid (PICF) and gingival tissues from healthy implants and peri-implantitis patients. The expression of RANKL in samples was tested by ELISA, Western blot and immunofluorescence staining. The production of RANKL in THP-1 macrophages stimulated with *P. gingivalis* was detected by qRT-PCR and Western blot. Then macrophages were pre-treated with neutralizing antibodies of Toll-like receptor 2 (TLR2) or lectin-type oxidized LDL receptor 1 (LOX-1) and inhibitors of TLR2, LOX-1 or Erk1/2 before *P. gingivalis* stimulation to evaluate the roles of TLR2, LOX-1 and Erk1/2 in RANKL production by qRT-PCR and Western blot.

Results: The protein level of RANKL was higher in PICF of peri-implantitis patients than healthy implants. We observed increased RANKL expression in *P. gingivalis* infected macrophages compared to controls. RANKL induced by *P. gingivalis* stimulation was mediated by TLR2 and Erk1/2 signaling pathway in THP-1 macrophages. LOX-1 is involved in TLR2 induced RANKL expression.

Conclusion: RANKL was involved in peri-implantitis, and regulated by TLR2, LOX-1 and Erk1/2 signaling against *P. gingivalis* infection. As the novel inflammation pathway triggers, TLR2 and LOX-1 which mediate RANKL production seems to be potential drug targets of peri-implantitis.

1. Introduction

Being an oral inflammatory disease, peri-implantitis plagues approximately 30% patients treated with dental implant which is one of the most frequently used treatments for patients' tooth replacement [1–4]. Many treatments for peri-implantitis have been described in former studies [5–7]. Unfortunately, there is still no one which has been proved to be highly effective against peri-implantitis in these therapeutic strategies.

In peri-implantitis, host and microbial factors lead to inflammation in supporting tissues surrounding implants and finally may lead to a complete loss of osseointegration [8,9]. As a bone loss biomarker, receptor activator of nuclear factor kappa-B ligand (RANKL) involved in loss of osseointegration induces osteoclastic bone erosion [10,11].

Being a member of the tumor necrosis factor (TNF) superfamily, RANKL, also known as the osteoprotegerin ligand (OPGL), affects the immune system, bone regeneration and remodeling [12,13]. On the myeloid lineage cells, RANKL acts as the key factor for osteoclast differentiation and activation by binding the receptor activator of nuclear factor kappa-B (RANK) [14,15]. As a dendritic cell survival factor in the immune system, RANKL leads to osteoclastogenesis and bone loss by regulating T cell-dependent immune response [16,17].

RANKL is also involved in pathogenesis of alveolar bone loss. Previous study showed that RANKL mediated by Toll-like receptor 2 (TLR2) and TLR4 exacerbates alveolar bone resorption induced by *Porphyromonas gingivalis* infection [18]. Serine dipeptide lipids of *P. gingivalis* promote TLR2/RANKL induced bone loss in periodontitis [19].

Up to now, no consensus about the difference of RANKL in the peri-implant crevicular fluid (PICF) between peri-implantitis and healthy implants has yet been reached in previous studies. Some studies showed that the protein level of RANKL was higher in peri-implantitis [20,21], and others did not show any significant difference [22,23]. Moreover, the regulatory mechanism of RANKL production upon peri-implantitis has not been good evaluated.

Therefore, we have twofold purpose in this study: to provide more data about the RANKL level in PICF from peri-implantitis patients and to reveal the regulatory mechanism of RANKL production upon *P.*

* Corresponding author.
E-mail address: dentistqianzhang@126.com (Q. Zhang).

https://doi.org/10.1016/j.intimp.2019.105956
Received 27 March 2019; Received in revised form 13 September 2019; Accepted 30 September 2019
Available online 23 October 2019

gingivalis infection.

2. Materials and methods

2.1. PICF collection

This study was approved by the ethics committee of the Affiliated Hospital of Qingdao University and conducted in accordance with the Helsinki Declaration. Each patient provided informed consent.

Ten healthy implant patients and ten peri-implantitis patients were studied. There are at least two dental implants in each patient. We investigated two implants of each patient. The criteria of diagnostic, inclusion and exclusion have been described in previous work from our laboratory [24]. PICF samples were collected by sterile Periopapers (Oraflow) which were placed in the sulcus of the gums and implants for 30 s. The second collection was in one minute later. The contents of PICF were eluted by 50 mM phosphate buffer contained 0.1 mM phenylmethylsulphonylfluoride for further tests.

2.2. Bacterial culture

P. gingivalis inoculum was provided by the Oral Laboratory of the Affiliated Hospital of Qingdao University, and grown in gifu anaerobic medium [25] broth at 37 °C under anaerobic conditions (5% H_2 and 5% CO_2 and 90% N_2). The bacteria were harvested after over night culture when optical density at 650 nm was 1 ($\sim 10^9$ colony forming units/ml), and washed twice before infecting human macrophages.

2.3. In vitro experiments

We purchased Human THP-1 cells from China Center for Type Culture Collection (Wuhan, China). Human macrophages used in our experiments were differentiated from THP-1 cells with 100 nM phorbol 12-myristate 13-acetate (PMA; Sigma) for 48-hours at a density of 1×10^6/well in 6-well plates. The cells were infected with live *P. gingivalis* at the multiplicity of infection (MOI) of 10, and harvested after 0, 4, 8, and 16-hours for qRT-PCR and after 0, 1/4, 1/2, 1, 4, and 16-hours for Western blot.

To study the roles of TLR2 and LOX-1 in *P. gingivalis* induced RANKL and LOX-1 production, TLR2 neutralizing antibody (R&D) (1 μg/ml), TLR2 inhibitor C29 (MCE) (50 μM), LOX-1 neutralizing antibody (R&D) (10 μg/ml), LOX-1 inhibitor Polyinosinic acid (Poly (I)) (Sigma) (250 μg/ml) were used to pre-treat THP-1 macrophages 2-hours before treated with *P. gingivalis* at 0, 8-hours for qRT-PCR and 0, 16-hours for Western blot. IgG (R&D), DMSO or sterile water was used for corresponding control.

To study the roles of Erk1/2 in *P. gingivalis* induced RANKL production, Erk1/2 inhibitor SCH772984 (SelleckChem) (10 μM) was used to pre-treat THP-1 macrophages 2-hours before treated with *P. gingivalis* at 0, 8-hours for qRT-PCR and 0, 16-hours for Western blot. DMSO was used for corresponding control.

2.4. Western blot

Bicinchoninic Acid Assay (Beyotime, China) was used to quantify total protein of PICF or THP-1 macrophages. Each well of a 10% polyacrylamide gel (BioRad) was loaded into total protein which was separated with electrophoresis and transferred to nitrocellulose membranes (Merck Millipore). Then anti-human RANKL (ProteinTech, 23408-1-AP, 1:1000), anti-human LOX-1 (ProteinTech, 11837-1-AP, 1:500) or anti-human β-actin (Cell Signaling, #12620, 1:1000) primary antibodies were used to incubate with the nitrocellulose membranes for overnight at 4 °C. And corresponding HRP-tagged secondary antibodies (Cell Signaling) were used to incubate with membranes for 2-hours at room temperature. Fusion Solo system (Vilber Lourmat) was used to develop Western blot with ECL substrate, and to quantify chemiluminescent signals showed by histograms.

2.5. Enzyme-linked immunosorbent assay (EILSA)

The EILSA protocol has been described in previous work from our laboratory [24]. RANKL primary antibody (1:1000; ProteinTech) was used for incubating with samples for 2-hours at room temperature on a medium speed shaker. Then anti-rabbit secondary antibody (1:8000; ProteinTech) was used to incubate for 1-hour. The substrate (Sigma) was added into wells at 37 °C for 30 min, followed by the optical density measure with a micro titer plate reader.

2.6. Immunofluorescence staining

The gingival tissues from peri-implantitis patients were used for RANKL immunofluorescence staining. The immunofluorescence protocol has been previously described [24]. The anti-human RANKL antibody (1:100; ProteinTech) was used to incubate slides. Then the AlexaFluor 488 (green) conjugated goat anti-rabbit IgG secondary antibody (1:1000; Cell Signaling) was added followed by a DAPI staining (1:500; Cell Signaling). Fluorescence microscopy was used to capture digital images.

2.7. RNA isolation and qRT-PCR

The mRNA levels of RANKL and LOX-1 in THP-1 macrophages were detected. PCR protocol has been described in previous publications [24]. The primer pair sequences were as follows: β-actin F-TGGCACC CAGCACAATGAA and R-CTAAGTCATAGTCCGCCTAGAAGCA; RANKL F-GCAGCATCGCTCTGTTCCTGTA and R-GCATGAGTCAGGTAGTGCTT CTGTG; LOX-1F-TCGGAAGCTGAATGAGAAATCC and R-CTTGCGGAC AAGGAGCTGA.

2.8. Statistical analysis

The statistical significance of the ELISA, RT-PCR and Western blot was determined with an unpaired, two-tailed Student's t-test. $P < 0.05$ was considered significant. All data were expressed as the mean ± SD.

3. Results

3.1. RANKL was involved in peri-implantitis

To investigate the RANKL levels in PICF, we tested protein levels by western blot and ELISA. Compare to healthy implants, the expression of RANKL in protein level were higher in PICF of partial peri-implantitis patients by Western blot (Fig. 1A). ELISA results (Fig. 1B) showed that RANKL protein level was increased in PICF of peri-implantitis patients ($P < 0.05$). RANKL expressed in partial gingival tissue of peri-implantitis patient by immunofluorescence staining (Fig. 1C). These results indicated that RANKL was involved in the nosogenesis of peri-implantitis.

3.2. RANKL elevated in P. gingivalis stimulated THP-1 macrophages

P. gingivalis is a momentous bacterial etiological agent associated with peri-implantitis [26–28]. To investigate the expression of RANKL in human macrophages upon *P. gingivalis* infection, we tested mRNA and protein expressions by qRT-PCR and western blot. The expression of RANKL in mRNA (Fig. 2A) and protein (Fig. 2B) levels was significantly increased after the *P. gingivalis* stimulation in THP-1 macrophages ($P < 0.05$, respectively), which indicated that RANKL increased by the stimulation of *P. gingivalis* in macrophages.

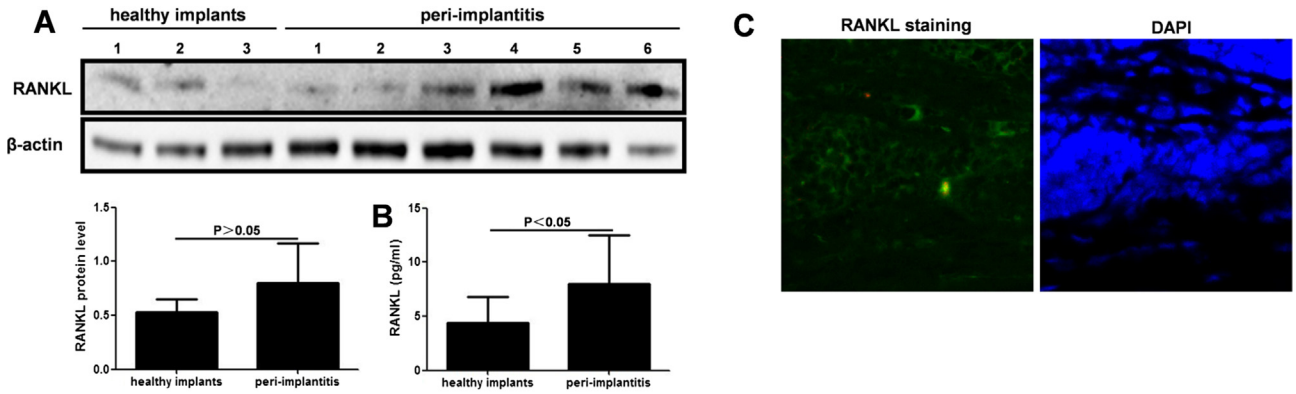

Fig. 1. RANKL was involved in peri-implantitis. RANKL levels in PICF from healthy implants and peri-implantitis patients (n = 10/group) were tested by Western blot (A) and ELISA (B). Immunofluorescence staining results showed that RANKL (green) expressed in partial gingival tissue of peri-implantitis patients (Magnification × 400) (C).

3.3. TLR2 induced RANKL production upon P. gingivalis infection

To determine the role of TLR2 in *P. gingivalis* induced RANKL expression of human macrophages, neutralizing antibody and inhibitor were used to blockade TLR2. With the pre-treatment of TLR2 neutralizing antibody or inhibitor before *P. gingivalis* stimulation, the expression of RANKL in mRNA (Fig. 3A and C) and protein (Fig. 3B and D) levels were significantly decreased in THP-1 macrophages ($P < 0.05$, respectively). These results indicated that RANKL production induced by *P. gingivalis* infection in human macrophages was induced by TLR2.

3.4. LOX-1 mediated RANKL production upon P. gingivalis stimulation

To determine the role of LOX-1 in *P. gingivalis* induced RANKL expression of human macrophages, neutralizing antibody and inhibitor were used to blockade LOX-1. With the pre-treatment of LOX-1 neutralizing antibody and inhibitor before *P. gingivalis* stimulation, the expression of RANKL in mRNA (Fig. 4A and C) and protein (Fig. 4B and D) levels were significantly decreased in THP-1 macrophages ($P < 0.05$, respectively). These results indicated that RANKL production induced by *P. gingivalis* infection in human macrophages was mediated by LOX-1.

3.5. LOX-1 is involved in TLR2 induced RANKL regulation against P. gingivalis

To determine the role of TLR2 in *P. gingivalis* induced LOX-1 expression of human macrophages, neutralizing antibody and inhibitor were used to blockade TLR2. With the pre-treatment of TLR2 neutralizing antibody or inhibitor before *P. gingivalis* stimulation, the expression of LOX-1 in mRNA (Fig. 5A and C) and protein (Fig. 5B and D) levels were significantly decreased in THP-1 macrophages ($P < 0.05$, respectively). These results indicated that LOX-1 is involved in TLR2 induced RANKL regulation against *P. gingivalis*.

3.6. Erk1/2 mediated P. gingivalis induced RANKL expression

Erk1/2 inhibitor SCH772984 was used to determine the role of Erk1/2 in *P. gingivalis* induced RANKL expression in THP-1 macrophages. With Erk1/2 inhibitor pre-treatment, the expression of RANKL in mRNA (Fig. 6A) and protein (Fig. 6B) levels were significantly decreased ($P < 0.05$, respectively). These results indicated that RANKL

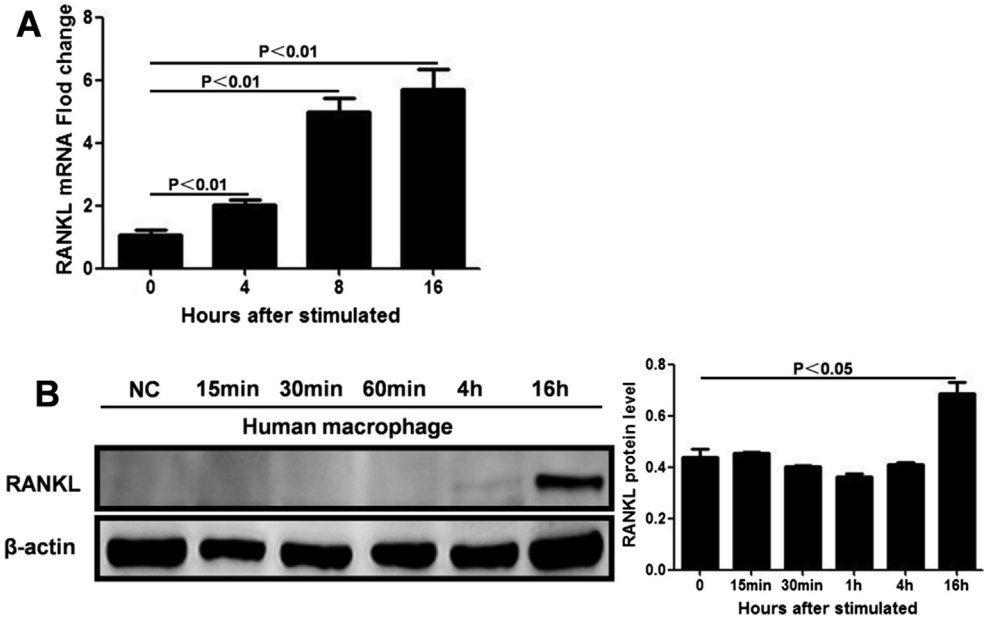

Fig. 2. RANKL elevated in *P. gingivalis* stimulated THP-1 macrophages. *P. gingivalis* was used to stimulate THP-1 macrophages at 0, 4, 8, and 16-hours for qRT-PCR (A), 0, 1/4, 1/2, 1, 4, and 16-hours for Western blot (B).

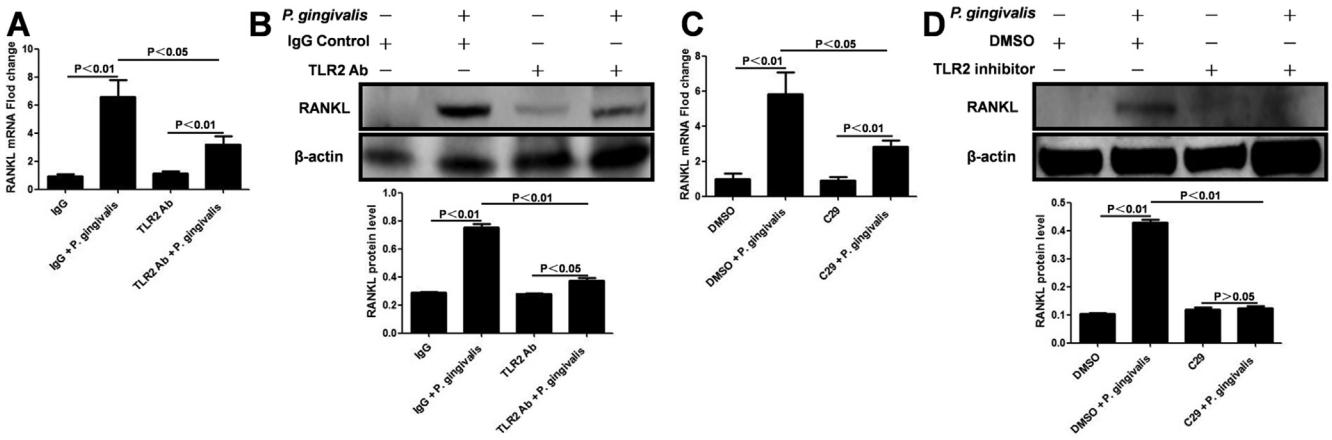

Fig. 3. TLR2 induced RANKL production upon *P. gingivalis* infection. TLR2 neutralizing antibody (1 μg/ml) and TLR2 inhibitor C29 (50 μM) were used to pre-treat THP-1 macrophages for 2-hours, following by *P. gingivalis* treatment for 16-hours. The expression of RANKL was analyzed by qRT-PCR (A and C) and Western blotting (B and D).

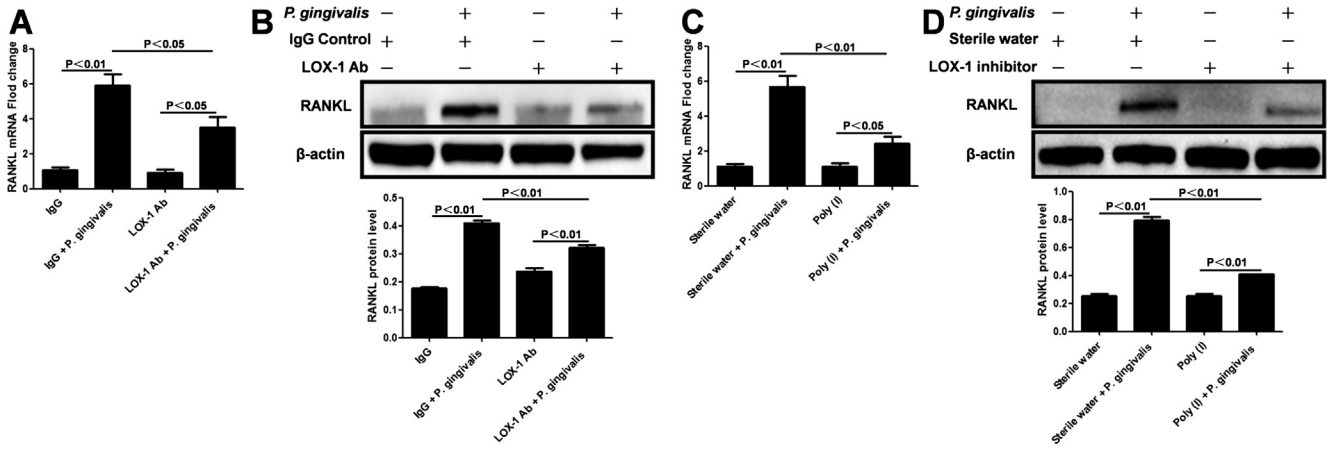

Fig. 4. LOX-1 mediated RANKL production upon *P. gingivalis* stimulation. LOX-1 neutralizing antibody (10 μg/ml) and LOX-1 inhibitor Poly(I) (250 μg/ml) were used to pre-treat THP-1 macrophages for 2-hours, following by *P. gingivalis* treatment for 16-hours. The expression of RANKL was analyzed by qRT-PCR (A and C) and Western blotting (B and D).

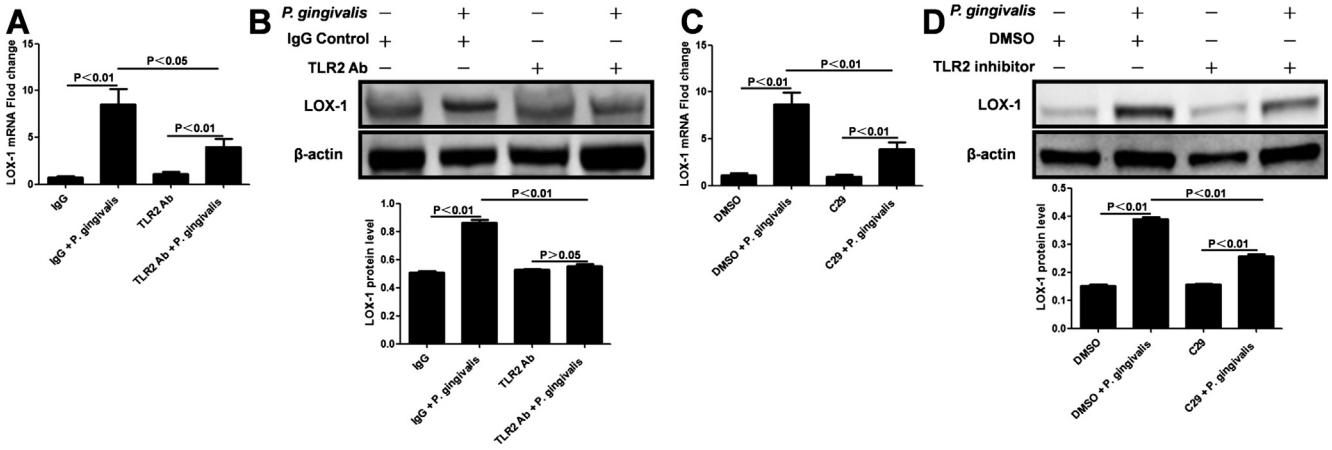

Fig. 5. TLR2 induced RANKL production upon *P. gingivalis* infection. TLR2 neutralizing antibody (1 μg/ml) and TLR2 inhibitor C29 (50 μM) were used to pre-treat THP-1 macrophages for 2-hours, following by *P. gingivalis* treatment for 16-hours. The expression of LOX-1 was analyzed by qRT-PCR (A and C) and Western blotting (B and D).

production induced by *P. gingivalis* stimulation in human macrophages was mediated by Erk1/2.

4. Discussion

Peri-implant, induced by a series of microbial factors including *P. gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Prevotellaintermedia*, has been defined as inflammatory lesions of the

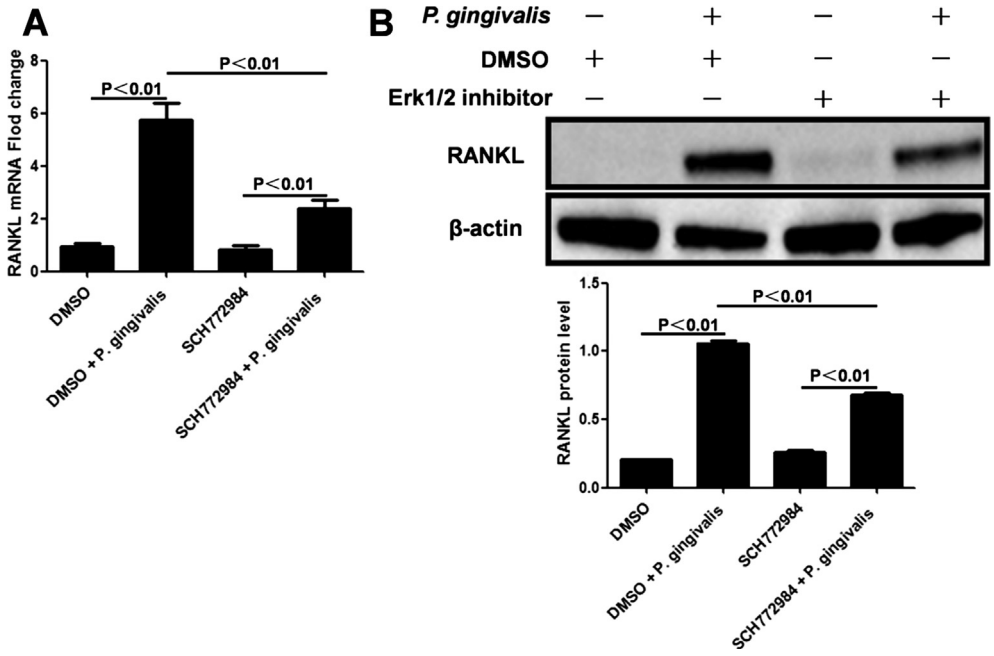

Fig. 6. Erk1/2 mediated *P. gingivalis* induced RANKL expression. Erk1/2 inhibitor SCH772984 (10 μM) was used to pretreat THP-1 macrophages for 2-hours, following by *P. gingivalis* treatment for 16-hours. The expression of RANKL was analyzed by qRT-PCR (A) and Western blotting (B).

surrounding hard and soft tissues [26–28]. In the study, we demonstrated that RANKL which is a bone loss biomarker participated in the pathogenesis of peri-implantitis. Rakic et al. [20,21] found that the protein level of RANKL in PICF from peri-implantitis patients was higher than healthy implants. Duarte et al. [22] found that there was no significant difference of RANKL in PICF from peri-implantitis patients and healthy implants. Our study showed that RANKL increased in PICF of partial peri-implantitis patients. In consideration of our following results that RANKL expression could induced by *P. gingivalis* stimulation, no consensus about RANKL in PICF was possiblely interrelated with the different microbial factors of dental peri-implantitis.

The enlarged blood vessels and infiltrated inflammatory cells including macrophages, lymphocytes and plasma cells occupied more than half of the connective soft tissue surrounding peri-implantitis [29]. The pathogenesis of periprostheticosteolysis is induced by severe macrophages derived inflammatory response and osteoclastogenesis. Based on this, we chose *P. gingivalis* stimulated THP-1 macrophages model to reveal the regulatory mechanism of RANKL in peri-implantitis. Wachi et al. [30] found that the stimulation from *P. gingivalis* LPS elevated RANKL expressions in MC3T3-E1 and GE-1 cells. Yamaguchi et al. [31] found that *P. gingivalis* oral infection increased alveolar bone loss and induced gingival IL-1β, IL-18, and RANKL expression in mice. Our results also reveal that RANKL elevated in *P. gingivalis* stimulated THP-1 macrophages.

As an important pattern recognition receptor, TLR2 is associated with osteoclastogenesis [32,33]. Kassem et al. [34] found that TLR2 induced RANKL expression to raise bone resorption and periosteal osteoclast development in Staphylococcus aureus septic arthritis. However, TLR2 is not the only pattern recognition receptor which is response for RANKL dependent bone resorption. Ohgi et al. [35] found that TLR2 induced LOX-1 up-regulation in mouse bone marrow cells to promote osteoclastogenesis. LOX-1 is a pattern recognition receptor belongs to the C-type lectin superfamily [36]. Oxidized low-density lipoprotein, apoptotic cells, bacteria and fungi are main ligands for LOX-1 [37,38]. Atherosclerosis induced by *P. gingivalis* is also correlated with LOX-1 [39,40]. Mai Nakayachi et al. [41] found that the expression of RANKL, IL-1β and prostaglandin E2 evoked by the inflammation were reduced in LOX-1 deficiency mice. Our previous study found that LOX-1 involves in IL-1β production and extracellular matrix breakdown in peri-implantitis [24]. In present study, our data demonstrated that down-regulation of TLR2 or LOX-1 reduced RANKL expression in *P. gingivalis* stimulated THP-1 macrophages. LOX-1 is involved in TLR2 induced RANKL regulation against *P. gingivalis*.

As the serine-threonine protein kinases group comprised by JNK, p38 and ERK1/2, MAPKs play important roles in host immune response and proinflammatory cytokines production [42,43]. Park et al. [44] found that 4-O-methylhonokiol suppressed RANKL-induced osteoclastogenesis by ERK1/2, AKT, and NF-kB pathways in bone marrow-derived macrophages. Yu et al. [45] found that RANKL gene expression was up-regulated by LPS induced ERK1/2 signaling pathway activation in MLO-Y4 cells. Our data indicated that the activation of Erk1/2 mediated TLR2 and LOX-1 induced RANKL expression in *P. gingivalis* stimulated THP-1 macrophages.

In summary, our present study indicated that RANKL was involved in peri-implantitis, and regulated by TLR2, LOX-1 and Erk1/2 signaling against *P. gingivalis* infection. As the novel inflammation pathway triggers, TLR2 and LOX-1 which mediate RANKL production seems to be potential drug targets of peri-implantitis.

Fundings

This study was supported by the National Natural Science Foundation of China (81500882) and Medical and Health Research Project of Shandong (2017WS491). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

[1] B. Schminke, F. Vom Orde, R. Gruber, H. Schliephake, R. Bürgers, N. Miosge, The pathology of bone tissue during peri-implantitis, J. Dent. Res. 94 (2) (2015) 354–361.
[2] T. Albrektsson, L. Canullo, D. Cochran, H. De Bruyn, "Peri-implantitis": a complication of a foreign body or a man-made "disease" facts and fiction, Clin. Implant. Dent. Relat. Res. 18 (4) (2016) 840–849.
[3] C.T. Lee, Y.W. Huang, L. Zhu, R. Weltman, Prevalences of peri-implantitis and peri-implant mucositis: systematic review and meta-analysis, J. Dent. 62 (2017) 1–12.
[4] R. Doornewaard, V. Christiaens, H. De Bruyn, M. Jacobsson, J. Cosyn, S. Vervaeke, W. Jacquet, Long-term effect of surface roughness and patients' factors on crestal bone loss at dental implants, a systematic review and meta-analysis, Clin. Implant.

Dent. Relat. Res. 19 (2) (2017) 372–399.
[5] S. Renvert, I. Polyzois, G.R. Persson, Treatment modalities for peri-implant mucositis and peri-implantitis, Am. J. Dent. 26 (6) (2013) 313–318.
[6] T. Jemt, A retro-prospective effectiveness study on 3448 implant operations at one referral clinic: a multifactorial analysis. Part II: Clinical factors associated to peri-implantitis surgery and late implant failures, Clin. Implant. Dent. Relat. Res. 19 (6) (2017) 972–979.
[7] B. Guler, A. Uraz, M. Yalım, S. Bozkaya, The comparison of porous titanium granule and xenograft in the surgical treatment of peri-implantitis: a prospective clinical study, Clin. Implant. Dent. Relat. Res. 19 (2) (2017) 316–327.
[8] E. Janska, B. Mohr, G. Wahl, Correlation between peri-implant sulcular fluid rate and expression of collagenase2 (MMP8), Clin. Oral. Investig. 20 (2) (2016) 261–1226.
[9] H. Kuula, T. Salo, E. Pirilä, A.M. Tuomainen, M. Jauhiainen, V.J. Uitto, L. Tjäderhane, P.J. Pussinen, T. Sorsa, Local and systemic responses in matrix metalloproteinase 8-deficient mice during Porphyromonas gingivalis-induced periodontitis, Infect. Immun. 77 (2) (2009) 850–859.
[10] F. Zhou, Y. Shen, B. Liu, X. Chen, L. Wan, D. Peng, Gastrodin inhibits osteoclastogenesis via down-regulating the NFATc1 signaling pathway and stimulates osseointegration in vitro, Biochem. Biophys. Res. Commun. 484 (4) (2017) 820–826.
[11] Y. Zhou, X. Guan, T. Liu, X. Wang, M. Yu, G. Yang, H. Wang, Whole body vibration improves osseointegration by up-regulating osteoblastic activity but down-regulating osteoblast-mediated osteoclastogenesis via ERK1/2 pathway, Bone 71 (2014) 17–24.
[12] N. Renema, B. Navet, M.F. Heymann, F. Lezot, D. Heymann, RANK-RANKL signalling in cancer, Biosci. Rep. 36 (4) (2016) e00366.
[13] R. Hanada, T. Hanada, V. Sigl, D. Schramek, J.M. Penninger, RANKL/RANK-beyond bones, J. Mol. Med. (Berl) 89 (7) (2011) 647–656.
[14] M. Honma, Y. Ikebuchi, Y. Kariya, H. Suzuki, Regulatory mechanisms of RANKL presentation to osteoclast precursors, Curr. Osteoporos. Rep. 12 (1) (2014) 115–120.
[15] W. Liu, X. Zhang, Receptor activator of nuclear factor-κB ligand (RANKL)/RANK/osteoprotegerin system in bone and other tissues, Mol. Med. Rep. 11 (5) (2015) 3212–3218.
[16] X. Chen, X. Zhi, P. Pan, J. Cui, L. Cao, W. Weng, Q. Zhou, L. Wang, X. Zhai, Q. Zhao, H. Hu, B. Huang, J. Su, Matrine prevents bone loss in ovariectomized mice by inhibiting RANKL-induced osteoclastogenesis, FASEB J. 24 (2017) 201700316R.
[17] L. Danks, N. Komatsu, M.M. Guerrini, S. Sawa, M. Armaka, G. Kollias, T. Nakashima, H. Takayanagi, RANKL expressed on synovial fibroblasts is primarily responsible for bone erosions during joint inflammation, Ann. Rheum. Dis. 75 (6) (2016) 1187–1195.
[18] J. Lin, L. Bi, X. Yu, T. Kawai, M.A. Taubman, B. Shen, X. Han, Porphyromonas gingivalis exacerbates ligature-induced, RANKL-dependent alveolar bone resorption via differential regulation of Toll-like receptor 2 (TLR2) and TLR4, Infect. Immun. 82 (10) (2014) 4127–4134.
[19] Y.H. Wang, R. Nemati, E. Anstadt, Y. Liu, Y. Son, Q. Zhu, X. Yao, R.B. Clark, D.W. Rowe, F.C. Nichols, Serine dipeptide lipids of Porphyromonas gingivalis inhibit osteoblast differentiation: relationship to Toll-like receptor 2, Bone 81 (2015) 654–661.
[20] M. Rakic, V. Lekovic, N. Nikolic-Jakoba, D. Vojvodic, A. Petkovic-Curcin, M. Sanz, Bone loss biomarkers associated with peri-implantitis: a cross-sectional study, Clin. Oral. Implants. Res. 24 (10) (2013) 1110–1116.
[21] M. Rakic, X. Struillou, A. Petkovic-Curcin, S. Matic, L. Canullo, M. Sanz, D. Vojvodic, Estimation of bone loss biomarkers as a diagnostic tool for peri-implantitis, J. Periodontol. 85 (11) (2014) 1566–1574.
[22] P.M. Duarte, A.C. de Mendonça, M.B. Máximo, V.R. Santos, M.F. Bastos, F.H. Nociti, Effect of anti-infective mechanical therapy on clinical parameters and cytokine levels in human peri-implant diseases, J. Periodontol. 80 (2) (2009) 234–243.
[23] F. Sarlati, M. Sattari, A.G. Gazar, A.N. Rafsenjani, Receptor activator of nuclear factor kappa B ligand (RANKL) levels in peri-implant crevicular fluid, Iran J. Immunol. 7 (4) (2010) 226–233.
[24] Che Chengye, Liu Jie, Xu. Ma Lei, Bai Na Huirong, Zhang Qian, LOX-1 is involved in IL-1β production and extracellular matrix breakdown in dental peri-implantitis, Int. Immunopharmacol. 52 (2017) 127–135.
[25] E. Park, H.S. Na, S.M. Kim, S. Wallet, S. Cha, J. Chung, Xylitol, an anticaries agent, exhibits potent inhibition of inflammatory responses in human THP-1-derived macrophages infected with Porphyromonas gingivalis, J. Periodontol. 85 (2014) e212–e223.
[26] C.F. Ferreira, J. Babu, A. Hamlekhan, S. Patel, T. Shokuhfar, Efficiency of nanotube surface-treated dental implants loaded with doxycycline on growth reduction of Porphyromonas gingivalis, Int. J. Oral Maxillofac. Implants 32 (2) (2017) 322–328.
[27] M.A. Stokman, A.J. van Winkelhoff, A. Vissink, F.K. Spijkervet, G.M. Raghoebar, Bacterial colonization of the peri-implant sulcus in dentate patients: a prospective observational study, Clin. Oral. Investig. 21 (2) (2017) 717–724.
[28] G. Schmalz, S. Tsigaras, S. Rinke, T. Kottmann, R. Haak, D. Ziebolz, Detection of five potentially periodontal pathogenic bacteria in peri-implant disease: a comparison of PCR and real-time PCR, Diagn. Microbiol. Infect. Dis. 85 (3) (2016) 289–294.
[29] H. Tang, N. Mattheos, Y. Yao, Y. Jia, L. Ma, P. Gong, In vivo osteoprotegerin gene therapy preventing bone loss induced by periodontitis, J. Periodontal. Res. 50 (4) (2015) 434–443.
[30] T. Wachi, T. Shuto, Y. Shinohara, Y. Matono, S. Makihira, Release of titanium ions from an implant surface and their effect on cytokine production related to alveolar bone resorption, Toxicology 327 (2015) 1–9.
[31] Y. Yamaguchi, T. Kurita-Ochiai, R. Kobayashi, T. Suzuki, T. Ando, Regulation of the NLRP3 inflammasome in Porphyromonas gingivalis-accelerated periodontal disease, Inflamm. Res. 66 (1) (2017) 59–65.
[32] M. Aizawa, K. Watanabe, T. Tominari, C. Matsumoto, M. Hirata, F.M.W. Grundler, M. Inada, C. Miyaura, Low molecular-weight curdlan, (1→3)-β-glucan suppresses TLR2-induced RANKL-dependent bone resorption, Biol. Pharm. Bull. 41 (8) (2018) 1282–1285.
[33] F. Cao, W. Zhou, G. Liu, T. Xia, M. Liu, B. Mi, Y. Liu, Staphylococcus aureus peptidoglycan promotes osteoclastogenesis via TLR2-mediated activation of the NF-κB/NFATc1 signaling pathway, Am. J. Transl. Res. 9 (11) (2017) 5022–5030.
[34] A. Kassem, C. Lindholm, U.H. Lerner, Toll-like receptor 2 stimulation of osteoblasts mediates staphylococcus aureus induced bone resorption and osteoclastogenesis through enhanced RANKL, PLoS One 11 (6) (2016) e0156708.
[35] K. Ohgi, H. Kajiya, K. Goto-T, F. Okamoto, Y. Yoshinaga, K. Okabe, R. Sakagami, Toll-like receptor 2 activation primes and upregulates osteoclastogenesis via lox-1, Lipids Health Dis. 17 (1) (2018) 132.
[36] S. Dunn, R.S. Vohra, J.E. Murphy, S. Homer-Vanniasinkam, J.H. Walker, S. Ponnambalam, The lectin-like oxidized low-density-lipoprotein receptor: a pro-inflammatory factor in vascular disease, Biochem. J. 409 (2) (2008) 349–355.
[37] Z. Wu, T. Sawamura, A.K. Kurdowska, H.L. Ji, S. Idell, J. Fu, LOX-1 deletion improves neutrophil responses, enhances bacterial clearance, and reduces lung injury in a murine polymicrobial sepsis model, Infect. Immun. 79 (7) (2011) 2865–2870.
[38] T. Shimaoka, N. Kume, M. Minami, K. Hayashida, T. Sawamura, T. Kita, S. Yonehara, LOX-1 supports adhesion of Gram-positive and Gram-negative bacteria, J. Immunol. 166 (8) (2001) 5108–5114.
[39] F. Liu, Y. Wang, J. Xu, F. Liu, R. Hu, H. Deng, Effects of Porphyromonas gingivalis lipopolysaccharide on the expression of key genes involved in cholesterol metabolism in macrophages, Arch. Med. Sci. 12 (5) (2016) 959–967.
[40] C.Y. Huang, C.M. Shih, N.W. Tsao, Y.W. Lin, C.C. Shih, K.H. Chiang, S.K. Shyue, Y.J. Chang, C.K. Hsieh, F.Y. Lin, The GroEL protein of Porphyromonas gingivalis regulates atherogenic phenomena in endothelial cells mediated by upregulating toll-like receptor 4 expression, Am. J. Transl. Res. 8 (2) (2016) 384–404.
[41] M. Nakayachi, J. Ito, C. Hayashida, Y. Ohyama, A. Kakino, M. Okayasu, T. Sato, T. Ogasawara, T. Kaneda, N. Suda, T. Sawamura, Y. Hakeda, Lectin-like oxidized low-density lipoprotein receptor-1 abrogation causes resistance to inflammatory bone destruction in mice, despite promoting osteoclastogenesis in the steady state, Bone 75 (2015) 170–182.
[42] C. Zhang, W. Wang, C. Liu, J. Lu, K. Sun, Role of NF-κB/GATA3 in the inhibition of lysyl oxidase by IL-1β in human amnion fibroblasts, Immunol. Cell Biol. 95 (10) (2017) 943–952.
[43] E.K. Kim, E.J. Choi, Compromised MAPK signaling in human diseases: an update, Arch. Toxicol. 89 (6) (2015) 867–882.
[44] K.R. Park, J.Y. Kim, E.C. Kim, H.M. Yun, J.T. Hong, RANKL-induced osteoclastogenesis is suppressed by 4-O-methylhonokiol in bone marrow-derived macrophages, Arch. Pharm. Res. (2017).
[45] K. Yu, Y. Ma, X. Li, X. Wu, W. Liu, X. Li, J. Shen, H. Wang, Lipopolysaccharide increases IL-6 secretion via activation of the ERK1/2 signaling pathway to up-regulate RANKL gene expression in MLO-Y4 cells, Cell Biol. Int. 41 (1) (2017) 84–92.