Role of DKK1 in Periodontitis and Innovative Strategy with its Neutralizing Antibody for Periodontitis Treatment

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Abstract

As host response can be hard to predict due to the complexity of pathogens in periodontitis, the efforts of work on etiology of periodontitis to eliminate inflammation seems complicated. To relieve bone resorption and prevent tooth loss eventually, we investigate the mechanism of bone homeostasis and target at the role of DKK1 in periodontitis. With abundance of evidence, we have the hypothesis that TNFα could induce high expression of DKK1, then block Wnt/β-catenin pathway, resulting in bone resorption even tooth loss in periodontitis. Anti-DKK1 antibody could reverse the damage and serve as an assisting therapy to relieve bone loss. If this hypothesis is clarified in the future, plenty of patients suffered from periodontitis will benefit from this antibody-mediated treatment.

Introduction

Periodontitis is a prevalent inflammation-ind ced disease. It can cause periodontal destruction and alveolar bone resorption, leading to tooth loss eventually (*Figure 1A*) 1. So far, periodontitis still remains the most common cause of tooth loss in the world [1]. And it can induce systematic problems as vascular endothelial dysfunction [2]. Thus, exploring an effective way to conquer this disease will be quite beneficial.

Periodontitis is an infectious disease involving a complex interaction between the oral microorganisms organized in a biofilm structure and the host immune response [1]. There can be an innate-only response, whilst others will need to invoke the inflammatory response and yet others will require the adaptive immune response to reduce or remove the microbial challenge [3]. And host response can be hard to predict due to the complexity of pathogens.

The most widely used treatment is physically removing the pathogenic bacterial-plaque biofilm by debridement, which appears as the gold standard for the treatment of inflammatory periodontitis for now [4,5]. However, it cannot fundamentally avoid inflammation relapse, because bacterial-plaque will always reattach, and stimulate inflammatory cascade constantly, especially for those patients with poor oral hygiene [6]. Conventional drug treatments, such as anti-inflammatory agents, have limited effects on reversing bone resorption, which is the crucial factor for tooth loss [7]. As new pathogens are suspected in the onset and progression of periodontitis, much more efforts of developing antibodies for pathogens in biofilm are expected [1]. In light of this, therapies that specifically target on bone turnover should be investigated and may serve as an assisting therapy.

A main hallmark of periodontitis pathogenesis is the imbalance of the osteoblast-osteoclast axis that is driven by periodontal inflammation, resulting in evident bone resorption by osteoclasts [8]. Wnt/β-catenin pathway is a key signaling for bone metabolism [9-13]. It promotes the differentiation of progenitor cells into osteoblasts to increase bone formation [14] and decrease bone resorption by reducing osteoclastogenesis [15-17]. As a member of Dickkopf (DKK) family, DKK1 is a natural inhibitor of Wnt/β-catenin pathway [18-21]. DKK1 expression can influence bone homeostasis and result in systematic bone diseases [22-25].

In periodontitis, inflammation uncouples bone formation from bone resorption via inflammatory mediators [26]. These mediators are able to induce the cascade of molecular events associated with extracellular matrix degradation and resultant tissue damage [7]. Among numerous inflammatory mediators, tumor necrosis factor-alpha (TNFα), a critical pro-inflammation cytokine [27,28] contributes significantly to the pathologic bone loss in periodontitis [26,29]. It is produced by activated macrophages as well as by many other connective tissue cells, such as synoviocytes and periodontal fibroblasts [7]. TNFα has been reported to act either directly on osteoclasts [30] or indirectly to induce osteoclast formation through the stimulation of RANKL production by osteoblasts [31].

Potential key role of DKK1 in the process of periodontitis

It has been reported that DKK1 appears to actively participate in joint remodeling in arthritis [22,32]. Similar with periodontitis, inflammation-induced bone loss occurs in rheumatoid arthritis (RA). Inflamm tory aspect causes destruction of joints and induces lytic lesions in the peri-atricular bone which is not adequately repaired by bone coupling [33].

Diarra et al. found that DKK1 protein expression was enhanced in synovial fibroblasts and increased in the serum of patients with RA compared with healthy controls. Furthermore, its expression could be induced by TNFα, while the blockade of DKK1 could reverse TNFα inhibitory effect on bone formation [32]. Using the anti-DKK1 antibody in human TNF transgenic (hTNFtg) mice not only led to a complete inhibition of bone erosions, but also promoted osteophyte formation in

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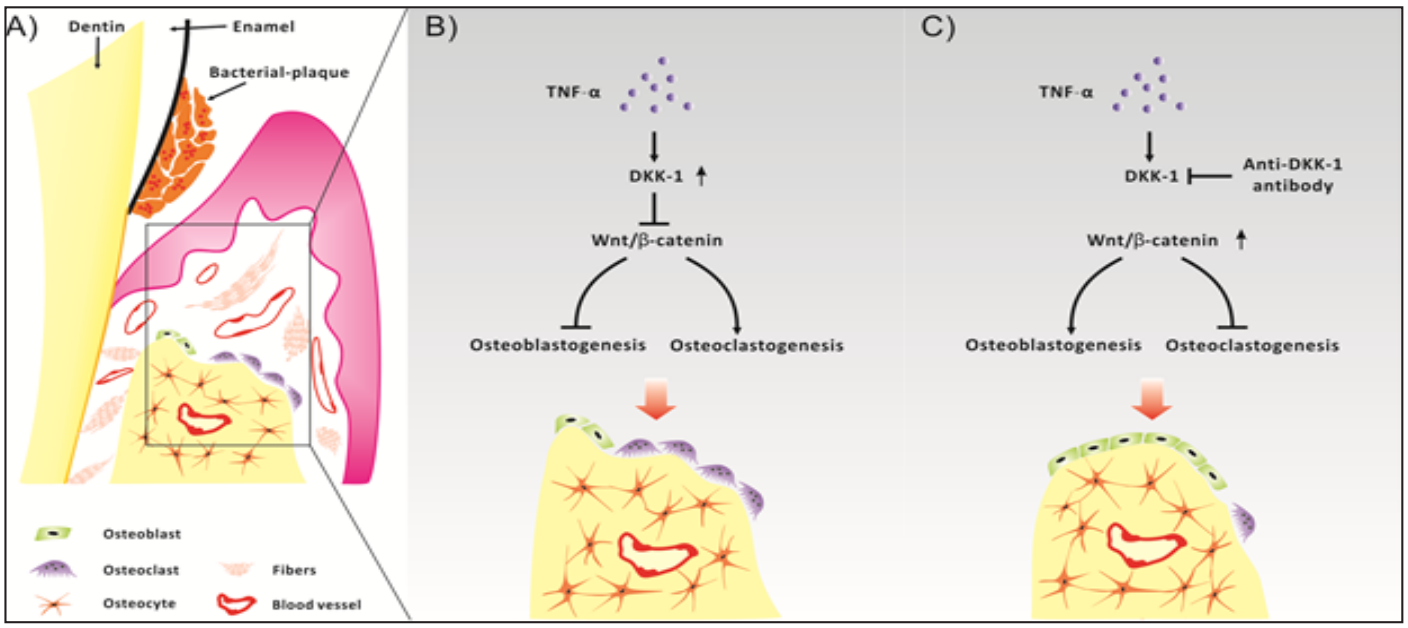

Figure 1. *A) The inflammation occur ed in the periodontium can cause collagen degradation and bone resorption, leading to tooth loss ultimately. B) We hypothesize that inflammatory bone loss in periodontitis may be at least partially owing to detrimental Wnt/β-catenin pathway caused by TNF-induced DKK1 overexpression. C) Local controlled-delivery of anti-DKK1 antibody in the treatment of periodontitis may be a new and powerful way for bone regeneration.*

sites prone to structural damage [32,34]. And the molecular mechanism of this new bone formation was associated with activation of the Wnt/β-catenin pathway [32].

Recently, evidence has been raised that the mRNA and protein levels of DKK1 were significantly increased in the gingival tissues of the chronic periodontitis when compared to the non-periodontitis group [35]. The induction of DKK1 by TNFα has been linked to increased bone loss in a mouse model of inflammatory arthritis and in human rheumatoid arthritis [33], supporting the present finding of the overexpression of TNFα in gingival tissue of periodontitis individuals. However, the data on the cellular and molecular mechanisms responsible for the increased levels of DKK1 in periodontal tissues is not provided [35]. A single study showed that the pharmacologic inhibition of SOST, another antagonist of Wnt/β-catenin pathway, restored the alveolar bone destruction following experimental periodontitis in rats by using a SOST neutralizing monoclonal antibody [36]. Interestingly, Blockade of DKK1 not only prevented impaired osteoblastogenesis, but also counteracted TNF-mediated SOST expression in differentiated osteoblasts in vitro and in vivo [35].

However, few researches involve promising therapeutic target of blocking DKK1. Given the critical role of TNFα in periodontitis, its ability to up-regulate expression of DKK1 in RA, and the high level of DKK1 expression in periodontitis, we propose that bone loss in periodontitis may partially due to downregulation of Wnt/β-catenin pathway by TNF-induced DKK1 overexpression (*Figure 1B*).

Innovative strategy with anti-DKK1 antibody for periodontitis treatment

One of the most interesting results from RA researches is that the "bone protective" effect by using anti-DKK1 antibody was achieved without altering the clinical signs of inflammatio , i ndicating a n u ncoupling o f inflammation and joint destruction. This directly inspires us to suppose that anti-DKK1 antibody may be a very promising agent for periodontitis treatment (*Figure 1C*).

Although periodontitis is a bacterially induced inflammatory disease [4], bacterial-plaque will always reattach after physical treatment, and stimulate inflammatory cascade constantly. On the other hand, once the inflammation occurs, numerous mediators and signaling molecules are involved in its development. However, the neutralization of DKK1 can protect bone and enhance new bone formation without altering the clinical signs of inflammation [32]. In other words, anti-DKK1 antibody can promote bone regeneration even if the inflammation cannot be eradicated entirely. This capacity undoubtedly has great value in periodontitis treatment.

Conclusion and Perspective

Integrated by evidence of role of Wnt/β-catenin pathway in bone physiology, induction of DKK1 by TNFα in RA and high expression of DKK1 in chronical periondontitis, we come to the hypothesis that TNFα can induce DKK1 to block Wnt/β-catenin pathway, resulting in bone loss in periodontitis. Based on this, we propose that specific anti-DKK1 antibody could serve as assisting therapy to prevent bone loss as well as systematic dysfunction caused by periodontitis.

Developing anti-DKK1 antibody provides us with a new direction to cure periodontitis. However, the cellular and molecular mechanism responsible for high level of DKK1 expression in periodontal tissues still remains unknown. In inflammatory environment, downregulation of the levels Wnt/β-catenin by treatment with DKK1 leads to activation of the noncanonical Wnt/Ca2+ pathway, resulting in the promotion of osteogenic differentiation in periodontal ligament stem cells (PDLSCs), which means that PDLSCs' ability of bone regeneration is awakened in inflammatory environment [37]. It seems DKK1-induced noncanonical Wnt pathway contributes

to relieve bone resorption indirectly, which is totally the opposite conclusion compared with above. It indicates that more details associating with DKK1 in the periodontal tissue remain to be discovered. The relationship between DKK1 and other molecules, cells and signaling pathways could be far more complicated beyond present knowledge. This hypothesis need to be clarified more thoroughly and deeply before it is used clinically.

Conflicts of inte est statement

None declared.

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References

1. Chaparro, Goncalves, Figueiredo, Faveri. Newly Identifie Pathogens Associated with Periodontitis A Systematic Review. *Journal of Dental Research.* 2014; **93**: 846-858.

2. Ahhijit NG. The implication of periodontitis in vascular endothelial dysfunction. *European Journal of Clinical Investigation* 2014.

3. Cyelee K, Denis FK. Host response in aggressive periodontitis. *Periodontology 2000*. 2014; **65**: 79-91.

4. Darveau RP. Periodontitis: A polymicrobial disruption of host homeostasis. *Nature Reviews Microbiology*. 2010; **8**: 481-490.

5. Connie LD. Periondontal Debridement Still The Treatment Of Choice. *Journal of Evidence-Based Dental Practice*. 2014; **14**: 33-41.

6. Offenbacher S, Barros SP, Beck JD. Rethinking periodontal inflammation *Journal of Periodontology.* 2008; **79**: 1577-1584.

7. Bartold PM, Cantley MD, Haynes DR. Mechanisms and control of pathologic bone loss in periodontitis. *Periodontology 2000.* 2010; **53**: 55-69.

8. Belibasakis & Bostanci Belibasakis GN, Bostanci N. The RANKL-OPG system in clinical periodontology. *Journal of Clinical Periodontology.* 2012; **39**: 239-248.

9. Nusse R. Wnt signaling in disease and in development. *Cell Research.* 2005; **15**: 28-32.

10. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Developmental Cell.* 2005; **8**: 739-750.

11. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. *Gene.* 2004; **341**: 19-39.

12. Baron R, Rawadi G. Targeting the Wnt/beta-catenin pathway to regulate bone formation in the adult skeleton. *Endocrinology.* 2007; **148**: 2635-2643.

13. Glass DA 2nd, Karsenty G. In vivo analysis of Wnt signaling in bone. *Endocrinology.* 2007; **148**: 2630-2634.

14. Hartmann C. Skeletal development-Wnts are in control. *Molecular Cell*. 2007; **24**: 177-184.

15. Glass DA, Bialek P, Ahn JD, Starbuck M. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Developmental Cell*. 2005; **8**: 751-764.

16. Glass DA, Karsenty G. Canonical Wnt signaling in osteoblasts is required for osteoclast differentiation. *Annals of the New York Academy of Sciences.* 2006; **1068**: 117-130.

17. Spencer GJ, Utting JC, Etheridge SL, Arnett TR. Wnt signalig in osteoblasts regulates expression of the receptor activator of NFkappaB ligand and inhibits osteoclastogenesis in vitro. *Journal of Cell Science*. 2006; **119**: 1283-1296.

18. Kubota T, Michigami T, Ozono K. Wnt signaling in bone metabolism. *Journal of Bone and Mineral Metabolism.* 2009; **27**: 265-271.

19. Glinka A, Wu W, Delius H, Monaghan AP. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature.* 1998; **391**: 357-362.

20. Bafico A, Liu G, Yaniv A, Gazit A. Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/ Arrow. *Nature Cell Biology.* 2001; **3**: 683-686.

21. Heath DJ, Chantry AD, Buckle CH, Coulton L. Inhibiting Dickkopf-1 (Dkk1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. *Journal of Bone and Mineral Research.* 2009; **24**: 425-436.

22. Daoussis D, Andonopoulos AP. The emerging role of Dickkopf-1 in bone biology: Is it the main switch controlling bone and joint remodeling? *Seminars in Arthritis and Rheumatism*. 2011; **41**: 170-177.

23. Li J, Sarosi I, Cattley RC, Pretorius J. Dkk1-mediated inhibition of Wnt signaling in bone results in osteopenia. *Bone.* 2006; **39**: 754-766.

24. MacDonald BT, Joiner DM, Oyserman SM, Sharma P. Bone mass is inversely proportional to Dkk1 levels in mice. *Bone.* 2007; **41**: 331-339.

25. Morvan F, Boulukos K, Clement-Lacroix P, Roman Roman S. Deletion of a single allele of the Dkk1 gene leads to an increase in bone formation and bone mass. *Journal of Bone and Mineral Research.* 2006; **21**: 934-945.

26. Graves DT, Li J, Cochran DL. Inflammation and uncoupling as mechanisms of periodontal bone loss. *Journal of Dental Research.* 2011; **90**: 143-153.

27. Lam J, Takeshita S, Barker JE, Kanagawa O. TNFαlpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *The Journal of Clinical Investigation.* 2000; **106**: 1481-1488.

28. Thomas MV, Puleo DA. Infection, inflam ation and bone regeneration: A paradoxical relationship. *Journal of Dental Research.* 2011; **90**: 1052-1061.

29. Garlet GP, Cardoso CR, Campanelli AP, Ferreira BR. The dual role of p55 tumour necrosis factor-alpha receptor in Actinobacillus actinomycetemcomitans-induced experimental periodontitis: Host protection and tissue destruction. *Clinical & Experimental Immunology.* 2007; **147**: 128-138.

30. Kobayashi K, Takahashi N, Jimi E, Udagawa N. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. *Journal of Experimental Medicine.* 2000; **191**: 275-286.

31. Schett G, Stach C, Zwerina J, Voll R. How antirheumatic drugs protect joints from damage in rheumatoid arthritis. *Arthritis & Rheumatology.* 2008; **58**: 2936-2948.

32. Diarra D, Stolina M, Polzer K, Zwerina J. Dickkopf-1 is a master regulator of joint remodeling. *Nature Medicine.* 2007; **13**: 156-163.

33. Walsh NC, Reinwald S, Manning CA, Condon KW. Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. *Journal of Bone and Mineral Research.* 2009; **24**: 1572-1585.

34. Heiland GR, Zwerina K, Baum W, Kireva T. Neutralisation of DKK1 protects from systemic bone loss during inflammation and reduces sclerostin expression. *Annals of the Rheumatic Diseases.* 2010; **69**: 2152-2159.

35. Marcelo HN, Cynthia N, Fa´bio LS, Tamires SM. Involvement of the Wnt-beta catenin signalling antagonists, sclerostin and dickkopf-related protein 1, in chronic periodontitis. *Journal of Clinical Periodontology*. 2014; **41**: 550-557.

36. Taut AD, Jin Q, Chung JH, Galindo-Mo-reno P. Sclerostin antibody stimulates bone regeneration after experimental periodontitis. *Journal of Bone and Mineral Research*. 2013; **28**: 2347-2356.

37. Na Liu, Songtao S, Manjing D, Liang T. Levels Wnt-beta Catenin Signaling Reduce Osteogenic Differentiation of Stem Cells in Inflammatory Microenvironments Through Inhibition of the Noncanonical Wnt Pathway. *Journal of Bone and Mineral Research*. 2011; **26**: 2082-2095.