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Review Article

Molecular mechanisms of *Porphyromonas gingivalis*-host cell interaction on periodontal diseases

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Summary *Porphyromonas gingivalis* (*P. gingivalis*) is a major oral pathogen and associated with periodontal diseases including periodontitis and alveolar bone loss. In this review, we indicate that two virulence factors, which are hemoglobin receptor protein (HbR) and cysteine proteases ''gingipains'', expressed by *P. gingivalis* have novel functions on the pathogenicity of *P. gingivalis*. *P. gingivalis* produces three types of gingipains and concomitantly several adhesin domains. Among the adhesin domains, hemoglobin receptor protein (HbR), also called HGP15, has the function of induction of interleukin-8 (IL-8) expression in human gingival epithelial cells, indicating the possibility that HbR is associated with *P. gingivalis*-induced periodontal inflammation. On bacteria-host cells contact, *P. gingivalis* induces cellular signaling alteration in host cells. Phosphatidylinositol 3-kinase (PI3K) and Akt are well known to play a pivotal role in various cellular physiological functions including cell survival and glucose metabolism in mammalian cells. Recently, we demonstrated that gingipains attenuate the activity of PI3K and Akt, which might have a causal influence on periodontal diseases by chronic infection to the host cells from the speculation of molecular analysis. In this review, we discuss new molecular and biological characterization of the virulence factors from *P. gingivalis*.

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Contents

1. Introduction

The periodontal diseases are known as prevalent oral disease and indicated asymptomatic diseases that are characterized by chronic inflammation of periodontal tissues, including gingival inflammation and alveolar bone resorption, and eventually tooth loss is provoked. Moreover, the diseases have been associated with systemic diseases: for example, cardiovascular disease, vascular disease, aspiration pneumonia, and diabetes [1—4]. The risk factors of periodontal diseases have indicated oral flora, environmental factors (e.g. smoking, stress, and diet), and host factors (e.g. immune system and genetic factors) [2]. Among the oral bacteria, *Porphyromonas gingivalis* has a close relationship with periodontal diseases [5,6]. *P. gingivalis* is a Gram-negative anaerobic and asaccharolytic bacterium that relies on the degradation of proteins and the generation of amino acids and peptides for the metabolic energy on its growth. *P. gingivalis* produces several known virulence factors, i.e., Lipopolysaccharide (LPS), fimbriae, proteases, and outer membrane vesicles [7—10]. It seems that *P. gingivalis* utilizes these factors not only for nutrient uptake and growth but also for avoidance from the host defense and survival in the host. Thereby, the virulence factors might be critical for the success of chronic infection of *P. gingivalis*.

2. Virulence factors of *P. gingivalis*

2.1. Lipopolysaccharide

LPS is one of the major virulence factors from this pathogen and has an ability to cause inflammation in the periodontal tissues [11]. *P. gingivalis* LPS causes a highly innate immune response through host receptors, which is toll-like receptor-2 (TLR-2) and TLR-4 on the host cell surface, leading to secrete interleukin-1 (IL-1), IL-6, IL-8, and TNF-α in host cells [12—15].

2.2. Capsule

P. gingivalis is known to synthesize the capsule which has also shown to be one of a variety of virulence factors of this organism [16,17]. Some studies have reported that encapsulated strains of *P. gingivalis* have more virulent than its non-encapsulated strains [16,18]. The capsule is associated with *P. gingivalis* escape from and reduction of host immune defense, promotes its survival in host cells [19,20], and induces serotype dependent cytokines expression in host cells [21].

2.3. Fimbriae

Fimbriae are a pivotal factor to adhere to host cell surface, extracellular matrix proteins, and coaggregation of oral bacteria and to invade into host cells [22,23]. *P. gingivalis* has major and minor fimbriae on its cell surface, and both fimbriae seem to contribute to establish the persistent infection and the development of periodontitis with expression of various cytokines, including IL-1, IL-6, and TNF-α [7,24—26]. Therefore, fimbriae might have an influence on the progression of periodontal disease.

2.4. Gingipains

P. gingivalis produces three cysteine proteases known as gingipains: arginine-gingipain A and B (RgpA and RgpB), and lysine-gingipain (Kgp), which play an important role in processing/maturation of its own cell surface proteins [27—30]. Meanwhile, gingipains have a detrimental effect on some biological activities of the host, which are degradation of components of the cell-to-cell contacts and detachment of epithelial cells from connective tissues of gingiva [31—33]. Gingipains have an adverse effect on healthy tissues via degradation of many human proteins including complement system proteins, cytokines, integrins, and collagen [9,34—36]. Recent reports have indicated that gingipains act as effectors for host cells and alter cellular signal transduction and cell physiological function [10,37,38]. Hence, gingipains from *P. gingivalis* are one of the most prominent virulence factors on periodontal diseases.

2.5. Adhesin domains

P. gingivalis has adhesin domains including hemmaglutinin (HGP44) and hemoglobin receptor protein (HbR) encoded

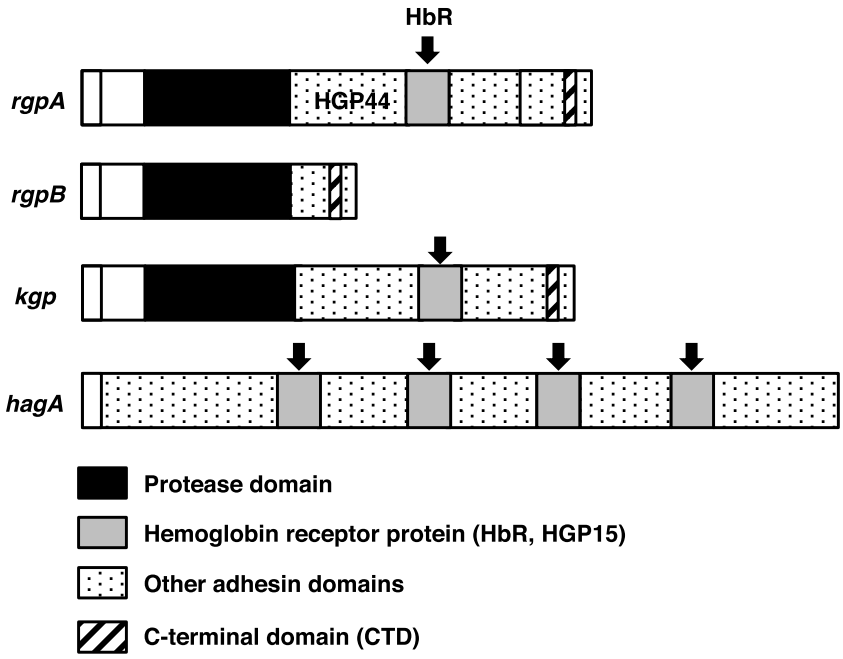

Figure 1 Structure of *rgpA*, *rgpB*, *kgp*, and *hagA* of *P. gingivalis*. Arrows indicate hemoglobin receptor protein (HbR, HGP15).

by *rgpA*, *kgp*, and *hagA*. Adhesin domains are formed by undergoing autocatalytic and intermolecular processing through the activity of gingipains, associate with gingipains as gingipains-adhesin complexes on bacterial cell surface and outer membrane vesicles [39]. Interestingly, although proteinase domains of RgpA and Kgp are divergent, their C-terminal adhesin domains containing HGP44 and HbR are very similar to each other (Fig. 1) [40—43]. HGP44 and HbR are also encoded by the hemagglutinin gene *hagA* [42,44,45]. These adhesin domains are required for maturation of bacterial cell-surface proteins including gingipains and fimbrilin [28,46], hemagglutination and hemolysis of erythrocytes [42,45,47], and binding of hemoglobin for heme acquisition of *P. gingivalis* [48,49], accordingly *P. gingivalis* can easily colonize in the gingival crevice and invade to the periodontal tissue.

2.6. Outer membrane vesicles

P. gingivalis produces outer membrane vesicles (OMVs) that mainly contain virulence factors such as LPS and gingipains which are associated with its pathogenicity [50,51]. OMVs are enriched in C-terminal domain proteins that are localized on the cell/vesicle surface through the type IX secretion system of *P. gingivalis* [30,52—54]. OMVs mediate bacterial coaggregation, and promote biofilm formation. Moreover, OMVs contribute to host interaction and colonization of *P. gingivalis* [8,51,55]. Thus, *P. gingivalis* releases a large number of its virulence factors into periodontal tissues in the form of OMVs.

3. Interleukin-8 (IL-8) production induced by ''Hemoglobin receptor (HbR)''

HbR binds hemoglobin and acts as hemophore to capture porphyrin and heme in need of iron for the growth of *P. gingivalis*. Fujimura et al. have reported that HbR interacted with host cells, and altered cellular signal transduction, following by inhibition of osteoclast differentiation from bone marrow macrophages [56]. We found the new function of HbR to induce expression of IL-8 from host epithelial cells via activation of cellular signal transduction [57]. Fujita et al. indicated that HbR remarkably increased expression of IL-8 in a dose-dependent manner, and revealed the mechanism by which HbR-induced IL-8 from gingival epithelial cells were associated with activation of p38MAPK and Erk1/2 using siRNAs and inhibitors. The relationship of transcription factors were determined activation of ATF-2, CREB, and NF-κB p65 by immunofluorescence and nuclear-translocation assay. Besides, it is likely that p38MAPK activated CREB, and Erk1/2 activated ATF-2 and NF-κB p65 (Fig. 2). This is the

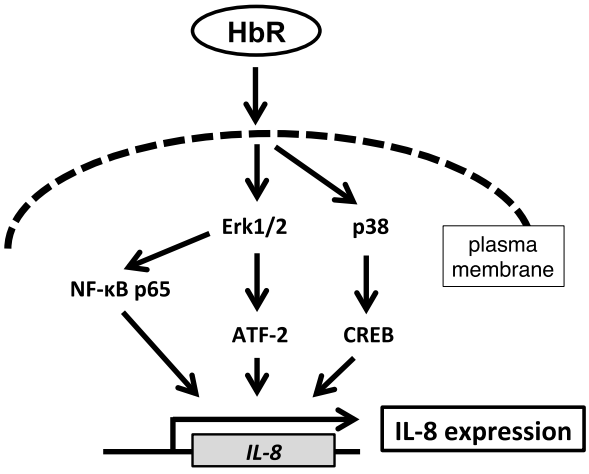

Figure 2 HbR induces IL-8 expression via activation of p38MAPK and Erk1/2, and of CREB, ATF-2 and NF-κB p65, respectively, in gingival epithelial cells.

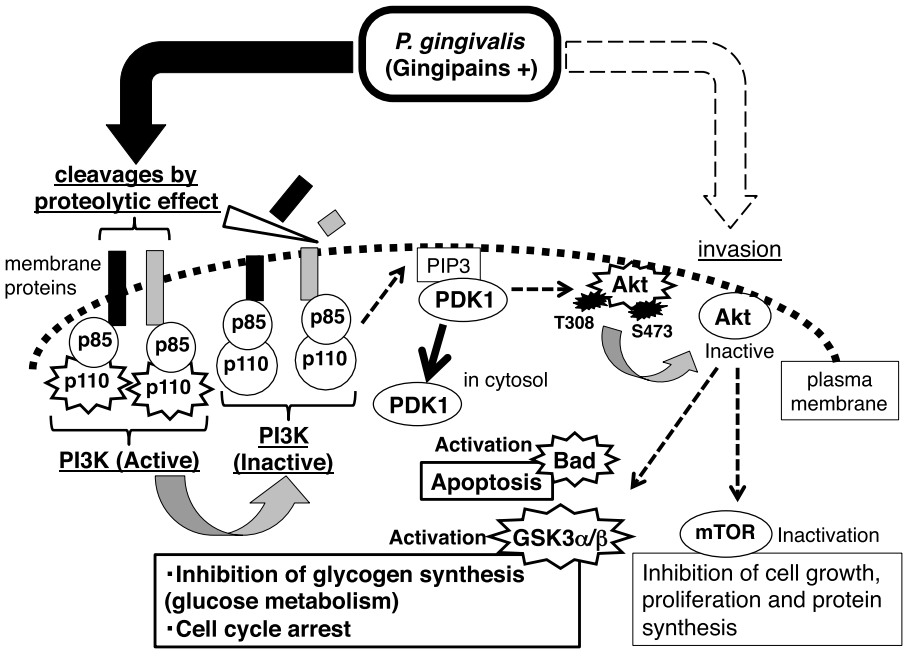

Figure 3 Molecular mechanisms of the attenuation of PI3K and Akt, and the dysregulation of PI3K/Akt signaling pathway by gingipains. Gingipains interact with membrane proteins associated with PI3K, and decrease in the activity of Akt, resulting in change the normal state of Akt downstream proteins, which are GSK3, Bad, and mTOR. These events are required for protease activity of gingipains independent of *P. gingivalis* invasion.

first study that adhesin domain has an ability to produce IL-8 from host cells. Interestingly, however, It has yet to be identified the receptor for HbR on epithelial cell membrane, which is significant for HbR to contact with host cells. Thus, HbR is considered as one of virulence factors of *P. gingivalis*, and there is potential involvement of HbR on etiology of periodontitis.

4. Effect of cysteine proteases ''Gingipains'' on PI3K/Akt signaling pathway

Gingipains have strong proteolytic activity, and facilitate growth and survival of the organism. However, they also serve as the virulence factor to damage the host cells and to establish long-term infection of *P. gingivalis* [28,58—61]. Here, we describe the possibility that gingipains have effects on host cell functions by disturbance of cellular signal transduction, which is phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway, on *P. gingivalis* infection. PI3K/Akt signaling pathway is one of the most important pathways for cell survival and growth, metabolism of glucose and protein synthesis in host cells [62—64]. There have been some reports between *P. gingivalis* and PI3K/Akt signaling pathway on bacteria-host cell crosstalk, indicating that *P. gingivalis* infection the activation of PI3K/Akt signaling pathway for host responses, such as expression of proteins, proinflammatory responses, and elimination of the organism [7,56,65]. However, we indicated the novel finding that gingipains attenuate the kinase activity of PI3K and Akt with their proteolytic activity independent of *P. gingivalis* invasion (Fig. 3) [10]. Using *P. gingivalis* wild-type strain and a gingipains-null mutant, PI3K and Akt were revealed the attenuation of these kinase activities by *in vitro* kinase assay and the decrease in the phosphorylation level of their down stream proteins/substrates such as GSK3, Bad, and mTOR. PDK1, which is upstream protein of Akt, was affected in the translocation to the plasma membrane by gingipains, and could not normally activate Akt and transmit the cascade of Akt signaling pathway. *P. gingivalis* can invade to host cells [66,67]. There is a possibility that this alteration of Akt signaling pathway is linked to the ability of *P. gingivalis* to invade to host cells. However, our results were not seemed to be independent of its invasion in gingival epithelial cells. Taken together, our studies showed novel molecular mechanisms by which gingipains affected PI3K/Akt signaling pathway and potentially disturbed cellular physiological functions regulated by PI3K and Akt, indicating that gingipains have a serious effect on the pathogenesis of periodontal diseases [33,59,68].

5. Perspective

Recent studies of HbR and gingipains have given new aspect as a virulence factor in pathogenicity of *P. gingivalis*. It is known that PI3K/Akt signaling pathway plays an important role in infectious diseases. Our study revealed that gingipains have the unique function as negative effector of this pathway, resulting in association with *P. gingivalis*-mediated destruction of periodontal tissues in periodontal diseases. Periodontal diseases are exhibited a typical symptom of inflammation in periodontal tissues. HbR is able to induce IL-8 production in gingival epithelial cells, indicating that HbR is involved in periodontitis as the virulence factor. Processing of RgpA, Kgp, and HagA by gingipains produces HbR, and three gingipain genes, which are *rgpA*, *rgpB*, and *kgp*, help the organism to accomplish long-term infection and colonization. Chronic infection leads the host to destroy the tissue, and develop periodontal diseases.

Conflict of interest

The authors have no conflict of interest related to this review.

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