

Special Article - Periodontics

Unrevealed Mechanisms of Bacterial Virulence in Periodontitis and Peri-Implantitis – Gingipains

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Abstract

As a part of dental plaque biofilm, *Porphyromonas gingivalis* is a major causative bacterium of chronic and aggressive periodontitis and play major role in development of peri-implant mucositis and peri-implantitis also. Arginine and lysine-specific proteases- gingipains HRgpA, RgpB and Kgp produced by *P. gingivalis* are fatal virulence factors for damaging host tissues. Periodontitis and peri-implantitis sites are both inflammatory lesions initiated by a bacterial infection and exist in a non-sterile environment with common cellular components and molecular pathways. These observations suggest that host modulatory approaches like inhibition of gingipains by various methods; those are successful for periodontitis will likely be applicable to peri-implantitis. For the reason, it is important to understand the structural and functional characteristics of gingipains. Present review is an attempt to explain these characteristics, which may be helpful in development of newer treatment strategies.

Keywords: HRgpA; RgpB; Kgp; Periodontitis; Peri-implantitis

Introduction

Chronic periodontitis is an infectious disease resulting in inflammation with in supporting tissues of the teeth, progressive attachment loss and bone loss. Generalized Aggressive Periodontitis (GAgP) is a chronic inflammatory disease of the periodontium, which is characterized by an accelerated rate of generalized alveolar bone resorption and, in severe cases, can lead to early tooth loss [1]. The term peri-implantitis was introduced in the 1980s to describe a destructive inflammatory process affecting the soft and hard tissues around osseointegrated implants, leading to the formation of a peri-implant pocket and loss of supporting bone (1st European Workshop on Periodontology, Ittingen, Switzerland). Despite rigorous clinical intervention strategies, recent reports suggest that up to 30% of adults in the U. S. over the age of 40 years have measurable periodontal bone loss [2]. Numerous studies implicate a variety of organisms with periodontal disease; however, *Porphyromonas gingivalis* remains the preeminent organism associated with periodontal and peri-implant diseases [3].

Gingipains are trypsin-like cysteine proteinases produced by *Porphyromonas gingivalis*. The *rgpA* gene of *Porphyromonas gingivalis* encodes the isoforms of the arginine-specific proteases HRgpA and RgpA(cat) and the membrane type mt-RgpA (cat), while *rgpB* encodes RgpB and the membrane type mt-RgpB. The lysine-specific protease Kgp is encoded by the *kgp* gene. Rgps (HRgpA and RgpB) and Kgp are specific for -Arg-Xaa- and -Lys-Xaa- peptide bonds, respectively [3].

Porphyromonas gingivalis derived gingipains play major role directly and indirectly at every stage of infection, not only in chronic and aggressive periodontitis, but also in occurrence of peri-implant mucositis and peri-implantitis by microbial attachment and colonization, acquisition of nutrients, evasion of host defence, and tissue invasion and dissemination suggested by Prof. Marzena

Dominiak at FDI Annual world Dental Congress-2016 in Poznan, Poland.

Rgps enhance vascular permeability through prekallikrein activation or direct bradykinin release in combination with Kgp. This Rgp action is potentially associated with gingival edema and crevicular fluid production. Rgps activate the blood coagulation system, leading to progression of inflammation and consequent alveolar bone loss in the periodontitis site [4]. Rgps also activate protease-activated receptors and induce platelet aggregation, which, together with the coagulation-inducing activity, may explain an emerging link between periodontitis and cardiovascular disease [4,5].

Kgp is the most potent fibrinogen/fibrin degrading enzyme of the three gingipains in human plasma, being involved in the bleeding tendency at the diseased gingiva. Gingipains stimulate expression of Matrix Metalloproteinases (MMPs) in fibroblasts and activate secreted latent MMPs that can destroy periodontal tissues. Gingipains degrade cytokines, components of the complement system and several receptors, including macrophage CD14, T cell CD4 and CD8, thus perturbing the host-defense systems and thereby facilitating sustained colonization of *P. gingivalis*. Gingipains are potent virulence factors of *P. gingivalis*, and in many regards their pathogenic activities constitute new mechanisms of bacterial virulence [6-8].

Structural characterization of Rgps and Kgp [9-13] (Figure 1,2).

Role of gingipains in host tissue damage and disease progression

The Arg- and Lys-proteinases have been either exposed at the surface (in the outer membrane) of the bacterium where they are able to come into contact with host cells and tissues or within the periplasmic space capable of being transported to the cell surface, and in outer membrane vesicles, which are sloughed from the outer membrane during growth [6,9] (Figure 3).

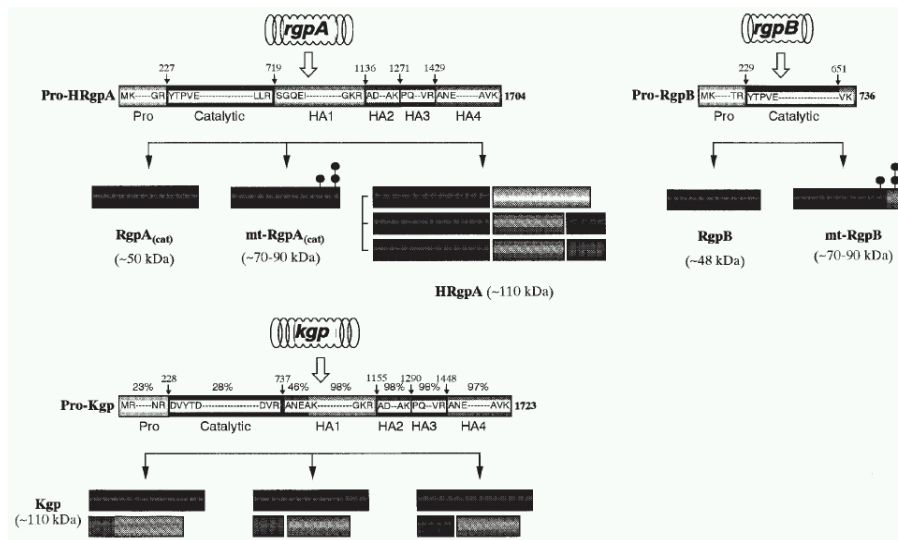


Figure 1: Isoform construction by proteolytic processing and assembly of the translated products of *rgpA*, *agpB* and *kgp* genes (HG66). Open arrows denote translation of gingipain genes to their proforms. Pro, profragment; catalytic domain; HA1, HA2, HA3, HA314, HA4, heaogglutinin/adhesion subdomains. Initial and endo amino acids of domains are shown. Small numbered arrows denote the cleavage site amino acid numbers and large arrows denote proteolytic processing and assembly of the mature enzyme complex, respectively. The domains with high homology are shown in the same pattern and percentage above Pro-Kgp columns denotes homology percentage of the Pro-Kgp domains for the corresponding Pro-HRgpA domains. Dots attached to mt-RgpA(cat) and mt-RgpB denote polysaccharide chains. Molecula weights of isoforms are shown in parenthesers (Modified from potempa et al. [11]).

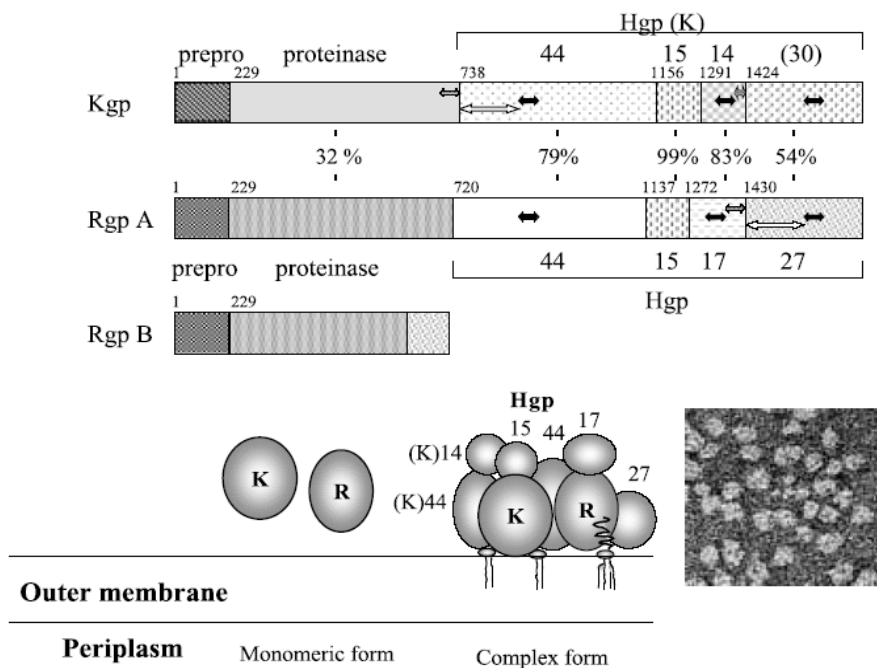


Figure 2: Schematic representation of the full length translation products from *rgp* and *kgp* genes and their structures. The numbers between RgpA and Kgp shows the identical percentages in the amino acids of each domain. The solid, striped, and open arrows represent the well-conserved regions. The lower right panel shows the electron micrograph of the purified membrane-associated gingipain complex.

Effect of Gingipain R and K on clotting system of host

Effect of Kgp fibrinogen lytic, leads to bleeding tendency during periodontitis. HrgpA potent thrombin producer, thrombin is not only invovled with blood clotting but also invovled with alveolar bone loss. Similarly Rgps increase Bradykinine production, which may lead to pain and alveolar bone loss. The gingipains are themselves, potent

non-fimbrial adhesins avidly binding several extracellular matrix proteins such as fibrinogen, fibronectin, laminin, and collagen type V [14]. They also apparently mediate a tight adherence to epithelial cells and gingival fibroblasts with Kgp being implicated as providing most of the binding. In gingival microvasculature, vascular endothelial thrombomodulin easily and pertinently degrade by Rgp B than RgpA

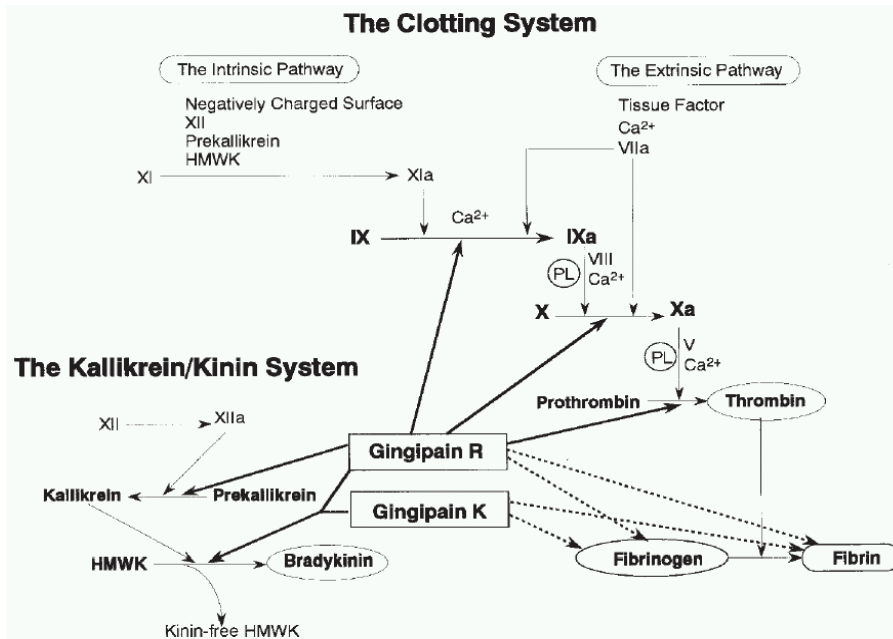


Figure 3: Effect of Gingipain R and K on clotting system of host.

and Kgp.

Gingipains exert a sequential action on which Rgps converts oxyhemoglobin to methemoglobin, which render the hemoglobin more susceptible to degradation by Kgp [15]. The occurrence of gingipains in large complexes is a very cleaver design to facilitate hemoglobin degradation and the capture of the released heme is accomplished with high affinity by hemagglutinin-adhesin-2. Gingipains may function as hemophore-like proteins; shuttling captured heme to a Hemoglobin Receptor (HmuR) in the outer membrane.

Gingipains degrades monocytes CD14, a major receptor for bacterial LPS, rendering monocytes hyporesponsive to bacterial LPS. In densely populated biofilm, gingipains as well as proteases released by other periodontopathogens can proteolytically inactivate cationic antimicrobial peptides to enable the survival of other bacterial species which are highly sensitive to them [16]. Degradation of cationic antimicrobial peptides also inactivates cationic antimicrobial peptides' ability to neutralize LPSs, which may lead to exacerbated, sustained production of proinflammatory cytokines [17].

P. gingivalis is resistant to killing by the human complement system. In a large part, this resistance is dependent on proteolytic activity of gingipains degrading different components of complement [18]. CXCL8 is an important signaling chemokine which is secreted in copious amounts by monocytes in response to infection and it serves to recruit neutrophils to the site of infection along a chemotactic gradient. Gingipains from *Porphyromonas gingivalis* play a significant role in induction and regulation of CXCL8 in THP-1 cells. Monocytes and neutrophils are sentinel cells of innate immunity and are found in abundance during periodontal infection [19]. THP-1 cells have been widely accepted and used as a surrogate for primary monocytes in biomedical research [20].

In addition, gingipains also contribute to proteolysis independent protection of *P. gingivalis* against complement-mediated lysis. This is achieved through the capture of the human complement inhibitor C4b-binding protein, thus, hindering deposition of the membrane attack complex on the *P. gingivalis* surface [21].

Gingipains efficiently degrade several extracellular matrix proteins *in vitro* gingipains can accomplish a lot more harm indirectly by disturbing the protease-protease inhibitor balance. In the case of human gingival fibroblasts, it was shown that matrix metalloprotease-1 expression was stimulated by Rgp activity [22]. Latent matrix metalloproteases can be directly activated by gingipains.

Conclusion

Natural or synthetic inhibitors of gingipains will lower the risk of periodontitis as well as peri-implantitis. The *fimA* type Ib genotype of *P. gingivalis* was found to play a critical role in the destruction of peri-implant tissue, suggesting that it may be a distinct risk factor for peri-implantitis [23]. Data suggest that immunization with RgpA stimulates the production of hemagglutinin domain-specific antibodies, which contribute to the prevention of *P. gingivalis*-mediated periodontal disease [24]. IgG and sIgA could be generated by immunization with *rgpA* DNA vaccine, which could significantly slow down bone loss in the experimental peri-implantitis canine model [25]. Vaccines, Gingipains inhibitors like chlorhexidine, Antibiotics like Tetracyclines are included in recent emerging treatment protocols against gingipains, but further human studies are necessary in this specific field as an adjunctive therapy in ameliorating chronic and aggressive periodontitis as well as peri-mucositis and peri-implantitis.

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