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Review

Novel function of *Porphyromonas gingivalis* gingipains in the PI3K/Akt signaling pathway

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ABSTRACT

Background: Porphyromonas gingivalis is s major oral bacterium closely associated with periodontal diseases including periodontitis and directly affects host cellular signaling. The phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway plays multiple roles in various cell functions including cell survival and glucose metabolism. In this review, we describe the effect of gingipains on the PI3K/Akt signaling pathway in *P. gingivalis* infection.

Highlight: Gingipains inactivate PI3K and Akt in gingival epithelial cells infected with *P. gingivalis*. These events occur independently of invasion of this organism into the cells and are required for the enzymatic activity of gingipains. Furthermore, 3-Phosphoinositide-dependent protein kinase-1 (PDK1) failed to translocate to the plasma membrane from the cytosol following PI3K inactivation.

Additionally, dephosphorylation of Akt downstream proteins, including glycogen synthase kinase 3 (GSK3), mammalian target of rapamycin (mTOR), and Bad, occurs in parallel with the dysregulation of PI3K/PDK1/Akt cascades.

Conclusion: This review describes the biological characterization of gingipains, which inactivate PI3K and Akt, and disorder the PI3K/Akt signaling pathway. Hence, gingipains may decrease cellular physiological functions, eventually disrupting the gingival epithelium and causing development of periodontal diseases.

Contents

1. Introduction

Periodontal diseases involve chronic inflammation in periodontal tissues and include periodontitis. The diseases are asymptomatic and associated with systemic diseases such as diabetes, cardiovascular disease, vascular disease, and aspiration pneumonia [1–3]. The risk factors influencing the development of periodontal diseases are oral flora, smoking, diet, and host factors (e.g.,

Abbreviations: PI3K, phosphatidylinositol-3 kinase; GSK3, glycogen synthase kinase 3; mTOR, mammalian target of rapamycin; PDK1, 3-phosphoinositide-dependent protein kinase 1; LPS, lipopolysaccharide

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immune system and genetic factors) [2,4]. *Porphyromonas gingivalis* is an oral bacterium closely associated with periodontal diseases [5,6]. This gram-negative anaerobic bacterium produces several known virulence factors, including gingipains, lipopolysaccharide (LPS), fimbriae, capsule, hemagglutinins, and outer membrane vesicles [7–13]. *Porphyromonas gingivalis* utilizes these factors to avoid host defenses, and successfully survives and colonizes in the host. Gingipains are the most potent factors among the virulence factors of *P. gingivalis*, and are responsible for persistent infection of *P. gingivalis* and damage to periodontal tissues [14–16].

The phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway has various functions in various host cells such as cell survival, proliferation, differentiation, endocytosis, metabolism, and host inflammatory responses [17,18]. Dysregulation of PI3K/Akt activity causes pathophysiological changes and leads to a variety of diseases, including cancer, type 2 diabetes, and infectious diseases [19–22]. *Porphyromonas gingivalis* stimulates the PI3K/Akt signaling pathway, which is associated with cell survival [23] and immune responses [24]. Furthermore, the PI3K/Akt signaling pathway is affected by virulence factors of *P. gingivalis* including LPS [25,26], fimbriae [27], and gingipains [28,29].

However, little is known regarding the significance of the PI3K/Akt signaling pathway in *P. gingivalis* infection. In this review, we focus on the effect of gingipains on the pathway and describe novel aspect of the pathogenicity of *P. gingivalis*.

2. Gingipains as virulence factors of *P. gingivalis*

Porphyromonas gingivalis produces various virulence factors as described in the Introduction. Among them, *P. gingivalis* contains three types of cysteine proteases, known as gingipains: arginine-gingipain A and B (RgpA and RgpB), and lysine-gingipain (Kgp) [30–32]. Gingipains have strong proteolytic activity, and are among the most potent virulence factors that damage host cells and establish long-term infection of *P. gingivalis* [30,33,34]. Gingipains can degrade complement system proteins, cytokines, integrins, and collagen [7,15,35]. Moreover, several studies indicated that gingipains alter cellular signal transduction and cell physiological function [8,36]. Thus, gingipains play a significant role in the colonization and survival of *P. gingivalis*, and influence the development of periodontitis.

3. Effect of *P. gingivalis* infection on the PI3K/Akt signaling pathway

The PI3K/Akt signaling pathway regulates cell survival and growth, glucose metabolism, and protein synthesis in host cells [37,38]. Some studies reported an interaction between *P. gingivalis* and the host PI3K/Akt signaling pathway, which is related to cell survival [23,39] and immune responses [24]. Among the virulence factors of *P. gingivalis*, LPS and fimbriae regulate immune reactions by activating PI3K and Akt [26,27]. Additionally, gingipains may modulate both cell survival and apoptosis via the PI3K/Akt cascade [29]. Recent reports indicated that *P.gingivalis* inactivates Akt in the liver, and modulates glycogen synthesis via GSK3, resulting in diabetes [40], and LPS induces Akt to inactivate mucin synthesis [41]. Thus, *P. gingivalis* activates and inactivates the PI3K/Akt signaling pathway, which has diverse functions in the pathogenicity of *P. gingivalis* infection.

4. Gingipains disturb the PI3K/Akt signaling pathway

Here, we describe the results of studies showing that gingipains suppress the activity of PI3K and Akt in gingival epithelial cells via extracellular interactions with their proteolytic activity, which is independent of *P. gingivalis* invasion into cells and intracellular activity. Gingival epithelial cells were challenged with *P. gingivalis* wild-type strain (WT-*Pg*) and a gingipains-null mutant (MT-*Pg*); WT-*Pg*, but not MT-*Pg*, attenuated PI3K and Akt. The downstream proteins of the PI3K/Akt cascade, GSK3, Bad, and mTOR, showed altered phosphorylation levels in parallel with PI3K/Akt inactivation in WT-*Pg*-infected cells. Importantly, examination of inhibitors of gingipains indicated that either Rgps (RgpA and RgpB) or Kgp decreased the activity of PI3K and Akt, suggesting that gingipains negatively regulate PI3K and Akt activity. Several inhibitors of endocytosis, pinocytosis, and lipid rafts-dependent endocytosis, including cytochalasin D, bafilomycinA1, and methyl-beta-cyclodextrin, respectively, induced inactivation of PI3K and Akt by gingipains, but inhibited *P. gingivalis* invasion. Moreover, gingipains cleaved membrane proteins coupled to PI3K p85, the regulatory subunit of PI3K, and disrupted complex formation between PI3K and membrane proteins, resulting in interference with homeostatic PI3K function and inactivation of PI3K and Akt. Thus, *P. gingivalis* invasion may not be strongly related to PI3K/Akt inactivation. No membrane proteins that bind PI3K p85 have been identified. Following inactivation of PI3K by gingipains, PDK1, a protein upstream of Akt, showed decreased plasma membrane localization in cells infected with WT-*Pg* compared to in uninfected cells and MT-*Pg*-infected cells. This indicates that PI3K could not convert phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-triphosphate. Additionally, Akt could not transmit information in the Akt signaling pathway. Therefore, gingipains interfere with the PI3K/PDK1/Akt cascade and disturb the Akt downstream proteins GSK3, mTOR, and Bad. However, our results differed from those of other studies and indicated that the PI3K/Akt signaling pathway is activated upon *P. gingivalis* infection. This may be because the cells used in the research were gingival epithelial cells, whereas other research groups utilized macrophages and gingival fibroblasts. Furthermore, the expression levels of receptors and adaptor proteins may differ between individuals. Experimental conditions, such as infection time, strains, virulence factors such as LPS and fimbriae, and culture conditions of *P. gingivalis* also differed between studies. Taken together, gingipains disturb the PI3K/Akt signaling pathway and potentially influence the cellular physiological functions regulated by this pathway (Fig. 1).

5. Conclusions

This review indicates that gingipains have novel functions as virulence factors in the pathogenicity of *P. gingivalis*. The PI3K/Akt signaling pathway is associated with infectious diseases. Gingipains altered the PI3K/PDK1/Akt cascade, disturbing the normal state of GSK3, mTOR, and Bad in gingival epithelial cells. Protease activity of gingipains is required for these events, which are not associated with *P. gingivalis* invasion and interactions within cells. Therefore, our findings indicate that gingipains induce the dysregulation of PI3K/Akt-regulated cellular functions, contributing to the destruction of epithelial barriers associated with periodontal disease development.

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