

# Proinflammatory Pathways Engaged by Oral Pathogen *Porphyromonas gingivalis* in Upregulation of Matrix Metalloproteinase-9 Expression in Periodontal Disease

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## Abstract

Matrix metalloproteinase-9 (MMP-9) is a potent endopeptidase implicated in a wide range of inflammatory and neoplastic diseases, including chronic periodontitis, a persistent oral mucosal inflammation attributed primarily to infection by *P. gingivalis*. Here, we review the signaling pathways engaged by *P. gingivalis* in controlling the processing and secretion of MMP-9. The induction in oral mucosal expression of MMP-9 by *P. gingivalis* relays primarily on its key endotoxin, LPS, engagement of TLR4 and the activation of MAPK, ERK and p38 cascades implicated in the stimulation of Rac1 and cPLA<sub>2</sub>. The ERK-mediated cPLA<sub>2</sub> phosphorylation plays an essential role in its membrane translocation with Rac1, while p38 localization with Rac1 promotes cPLA<sub>2</sub> activation and the induction in MMP-9. Moreover, the induction in MMP-9 secretion by the LPS and the modulatory influence of peptide hormone, ghrelin, occur at the level of MMP-9 processing between ER and Golgi, with the involvement of factors that control secretory cargo sorting, Arf1 GTPase and PKD2. The secretion of MMP-9, furthermore, occurs in concert with the changes in stability dynamics of microtubules (MTs), which affect the Golgi localization of Arf1 and the recruitment and activation of PKD2. The induction in MMP-9 secretion by LPS is accompanied by the enhancement in MT stabilization and  $\alpha$ -tubulin phosphorylation on Ser, while the MT destabilization associated with the modulatory influence of ghrelin, is manifested by  $\alpha$ -tubulin phosphorylation on Tyr. Thus, the factors capable of affecting MT dynamics and MMP-9 secretion present a tempting target for the therapeutic intervention in the treatment of chronic periodontitis.

## Keywords

*P. gingivalis*, Periodontal Disease, Inflammatory Response, LPS,

## 1. Introduction

*Porphyromonas gingivalis*, a rod-shaped, Gram-negative anaerobe together with two other oral bacterial species colonizing the oral cavity, *Treponema denticola*, and *Tannerella forsythia*, form so called “red bacterial complex” recognized as the primary etiological cause of periodontitis, a chronic inflammatory disease that leads to progressive destruction of teeth-supporting tissue and is the major cause of adult tooth loss [1] [2] [3] [4]. A growing body of evidence, moreover, indicates that periodontitis affects the course and pathogenesis of a number of systemic diseases such as cardiovascular disease, infective endocarditis, atherosclerosis, bacterial pneumonia, diabetes mellitus, leukemia, and low birth weight [5] [6] [7]. There are also strong supporting data linking periodontitis and *P. gingivalis* infection to rheumatoid arthritis, and the progression and metastasis of oral squamous cell carcinoma [8] [9] [10] [11].

Indeed, *P. gingivalis*, by far the most prominent member of the bacterial flora found in periodontal packets of people with gum disease [12], has to its disposal an impressive array of virulence factors that provoke the inflammatory response of the host tissue [13] [14]. These include its cell surface major (FimA) and minor (Mfa1) fimbriae proteins, elaborated arginine and lysine specific cysteine proteinases, referred to as gingipain R and gingipain K [15] [16], collagenases, capable of extracellular matrix proteins degradation and activation of the host matrix metalloproteinases [17] [18] [19], and the secreted sulfatases directed towards cell membrane glycosphingolipids as well as proteoglycans of extracellular matrix [3] [20]. However, the virulence factor of particular significance to the pathogenic action of *P. gingivalis* leading to the development of periodontitis is its cell-wall lipopolysaccharide (LPS) [21] [22].

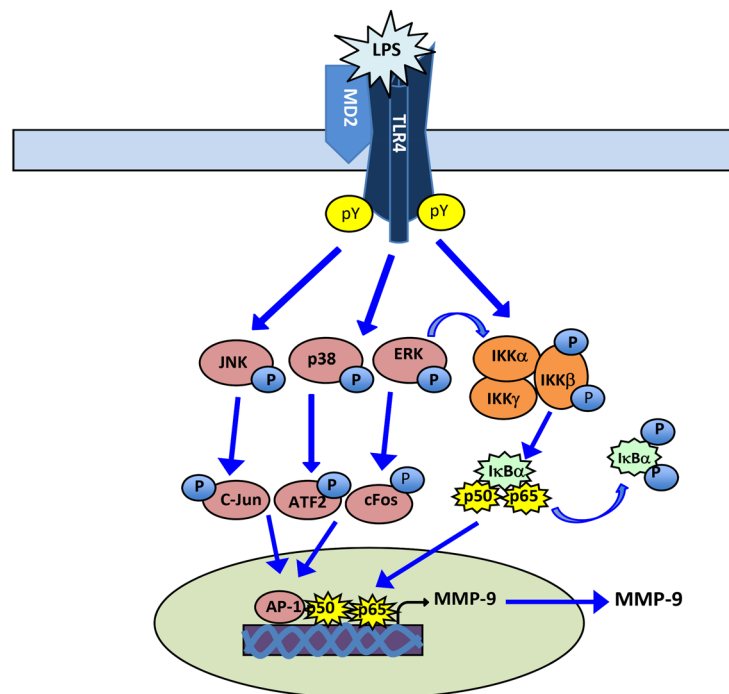
In general, the bacterial LPS consists of membrane anchored hydrophobic domain, referred to as lipid A, non-repeating core oligosaccharide region, and a distal polysaccharide structure referred to as O-antigen chain. The lipid A region is composed of phosphorylated (1,6)-linked diglucosamine backbone with four to seven fatty acyl chains ranging from 10 to 16 carbon units attached to it. Of these, four acyl chains are directly linked to the glucosamine backbone, and the remaining being attached to the hydroxyl groups of the lipid chains [23] [24]. The non-repeating core oligosaccharide region is rich in heptose and KDO (keto-deoxyoctulosonate), and coupled to the O-specific highly heterogeneous set of carbohydrate chains extending from the cell surface [23] [24] [25]. The endotoxin and the immunological properties of LPS reside mainly in the heterogeneous acylation character of lipid A domain, which in different bacterial species shows considerable variation in the acylation pattern and the number of phosphate groups, while the core and O-specific regions of LPS appear to have only minor role in the immune recognition [23] [24] [26] [27] [28].

Investigations into the events associated with oral mucosal responses to *P. gingivalis* LPS demonstrated a marked increase in epithelial cell apoptosis and proinflammatory cytokine expression, up-regulation in endothelin-1 (ET-1) and TNF- $\alpha$  release, excessive nitric oxide (NO) and prostaglandin (PGE2) generation, and the elevated levels of matrix metalloproteinase-9 (MMP-9) production [18] [21] [29] [30] [31] [32] [33]. Hence, considering that the elevated levels of MMP-9, along with the increased proinflammatory cytokine production are implicated in pathogenesis of chronic periodontitis, in this article we review data on the signaling pathways engaged by periodontopathogen, *P. gingivalis*, in controlling the processing and secretion of MMP-9.

## 2. *P. gingivalis* and TLR4 Activation

### 2.1. MMP-9 in Inflammatory Responses to Bacterial LPS

The mechanism underlying the inflammatory processes elicited by *P. gingivalis* in oral mucosa relies on the interaction of its LPS with Toll-like receptor-4 (TLR4). The LPS triggered stimulation of TLR4 signaling pathways (Figure 1) leads to activation of I $\kappa$ B-kinase complex (IKK) and up-regulation in mitogen-activated protein kinases (MAPKs), which trigger the activation of transcriptional factors that exert control over a wide range of proinflammatory mediators, including MMP-9 [32] [33] [34].



**Figure 1.** Signaling path ways and transcription factors involved in oral mucosal secretion of MMP-9 in responses to *P. gingivalis* LPS. Ligation of TLR4 by the LPS results in the activation of IKK complex and MAP kinases, ERK, p38 and JNK, and triggers the nuclear translocation of transcription factors, AP-1 and NF- $\kappa$ B, involved in the induction of MMP-9 gene transcription. pY, phosphotyrosine; P, phosphate.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases implicated in the remodeling of extracellular matrix under normal physiological conditions, as well as during angiogenesis, wound healing, tumor metastasis, and a wide range of degenerative and inflammatory diseases, including oral cancer, Sjogren's syndrome, rheumatoid arthritis, oral lichen planus, and chronic periodontitis [3] [19] [34]-[39]. These multidomain enzymes are composed of the active site and the catalytic metal binding domain which is kept inactive by N-terminal pro-peptide domain (34). The catalytic domain contains the zinc binding site, a calcium ion and conserved methionine. These are essential for maintenance of MMP three-dimensional structure and enzyme activity. The unique amino acid sequences within the active site are of importance for the enhancing of catalytic efficiency towards gelatin and peptide substrates [34]. The pro-peptide domains of MMPs contain the cysteine switch sequence, which ligates the catalytic zinc to maintain the enzyme inactive. Activation of the enzyme requires either proteolytic processing of the pro-peptide domain or steric disruption of the pro-peptide conformation [34].

While the main function of MMPs is the regulation of cell matrix composition, the enhanced production of MMPs, and MMP-9 in particular, is associated with host reaction to microbial and fungal infections [40] [41], as well as characterizes inflammatory response to lipopolysaccharide (LPS) of Gram-negative bacteria in several different cell systems [25] [42] [43] [44]. The elevated levels of MMP-9, elicited in oral mucosa in response to *Porphyromonas gingivalis* and its key endotoxin, LPS, furthermore, are directly responsible for persistent mucosal inflammation that leads to periodontal lesions and progressive destruction of teeth-supporting tissue, including bone loss [4] [19] [38] [41] [44]. There are also strong indications that enhanced levels of MMP-9 in serum of periodontal disease patients may contribute to extra-oral sequela, such as a decrease in cardiac function and cardiovascular disease [3] [4] [17]. Interestingly, the association between elevated serum content of MMP-9 and the increased risk of cardiovascular disease was also observed in patients with *H. pylori*-associated gastritis/diseases, and a decrease in the mucosal level of MMP-9 occurred following successful *H. pylori* eradication therapy [45] [46]. There are strong indications that the elevated levels of MMP-9 contribute directly to the inflammatory process by proteolytic tissue destruction, recruitment of inflammatory cells, and the generation of inflammatory signals. Indeed, the induction in MMP-9 expression is observed in wound healing, whereas the inhibition of MMP-9 has been linked to aberrant re-epithelization [9] [34] [35]. Moreover, MMPs, including MMP-9, are involved in extracellular matrix and basement membrane degradation and the activation of factors that perpetuate the inflammatory process [35].

Although the rate-limiting step in MMP-9 regulation is gene transcription, the expression and activity of this heavily glycosylated, 92 kDa, endopeptidase remains also under the influence of posttranslational processing, proenzyme activation, and the inhibition by the family of endogenous tissue inhibitors of me-

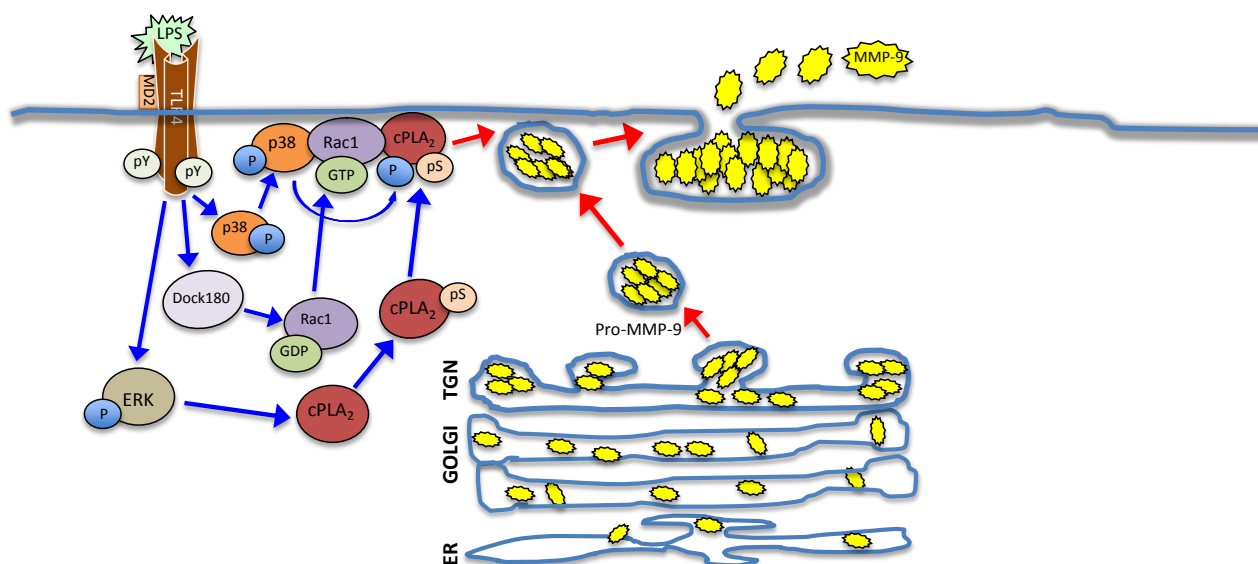
talloproteinases (TIMPs) [34] [42]. This multifaceted control assures the low MMP-9 expression in normal tissue, and its rapid release and activation in response to inflammatory stimulus such as LPS [43]. Therefore, therapies aimed at decreasing the MMP-9 expression and its enzymatic activity by the use of specific MMP inhibitors could provide much needed options in the treatment of inflammatory diseases, including chronic periodontitis. A positive development along these lines is the successful use of modified tetracycline derivative in the treatment of periodontitis [34].

## 2.2. LPS-Induced TLR4 Activation and MMP-9 Expression

Studies into the processing of MMP-9 in response to LPS stimulation indicate that MMP-9 expression is tightly controlled at the transcriptional level by MAPK cascade, including ERK, JNK, and p38, which along with IKK exert their influence over transcriptional factors AP-1 and NF- $\kappa$ B activation [47] [48]. Moreover, these responses to *P. gingivalis* are mediated through the LPS triggered TLR4 activation [18] [33].

Indeed, the available data, suggests that MAPK cascade activation upon LPS stimulation plays an important role in the regulation of intracellular trafficking, membrane translocation, and the release of secretory products [43] [49] [50]. The LPS-induced p38 activation and its recruitment to the cytosolic aspect of the membrane-localized Rac1 GTPase has been linked to disintegrin-metalloprotease ADAM17 activation [32] [49], and the up-regulation in MAPK and Rac activation was observed in association with LPS-induced pulmonary inflammation and TLR4-stimulated phagocytosis [51] [52]. There are also strong indications that ERK activation upon LPS stimulation plays an important role in the phosphorylation of cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) that facilitates the enzyme translocation from cytosol to membrane to gain access to phospholipid substrates [33] [50]. Moreover, further up-regulation in cPLA<sub>2</sub> activation has been reported to occur with the involvement of Rac1/p38 complex [53] [54]. Consistent with these facts are the data pointing to the role of MAPK signaling in targeting of cPLA<sub>2</sub> to intracellular membranes involved in secretory cargo processing, such as the Golgi vesicle formation and the membrane-vesicle fusion events that affect the secretory cargo release [53] [55] [56].

Therefore, taking into account the evidence that MMP-9 undergoes extensive processing in the Golgi network and the fact that oral mucosal inflammatory responses to *P. gingivalis* are characterized by the rapid rise in MMP-9 release, we investigated the nature of factors involved in the processing of signaling cascade initiated by the LPS [18]. As summarized in **Figure 2**, our findings demonstrated that *P. gingivalis* LPS-elicited induction in the salivary gland acinar cell MMP-9 release is associated with MAPK, ERK and p38 activation, and occurs with the involvement of Rac1 and cPLA<sub>2</sub>). Moreover, the release of MMP-9 involves ERK-mediated phosphorylation of cPLA<sub>2</sub> on Ser<sup>505</sup> that is essential for its membrane translocation with Rac1, and that this process requires p38 activation. Apparently, the activation and membrane localization of p38 with Rac1-GTP



**Figure 2.** Diagram of the pathways involved in *P. gingivalis* LPS-elicited induction in salivary gland acinar cell MMP-9 release. Binding of the LPS to TLR4 triggers up-regulation in ERK and p38 MAPK activation, as well as induces the pathway of GEF, Dock180-mediated GTP-Rac1 formation and its membrane translocation. This in turn, promotes the membrane localization of pp38 with the Rac1-GTP, while pERK is involved in the phosphorylation of cPLA<sub>2</sub> on Ser<sup>505</sup>, which prompts its recruitment to the Rac1/p38 complex, and the induction in cPLA<sub>2</sub> activation. The cPLA<sub>2</sub>, by acting on membrane phospholipids, affects the membrane fusion events, and leads to the induction in MMP-9 release. P phosphate, pS phosphoserine, pY phosphotyrosine.

plays a pivotal role in cPLA<sub>2</sub>-dependent induction in MMP-9 release [18]. Collectively, the findings infer that *P. gingivalis* LPS-induced up-regulation in the acinar cell MMP-9 release requires ERK-dependent recruitment of cPLA<sub>2</sub> to the membrane localized Rac1/p38 complex.

### 3. Factors Affecting MMP-9 Processing

#### 3.1. ADP-Ribosylation Factors

MMP-9 is a highly glycosylated endopeptidase, consisting of both N- and O-linked carbohydrate chains, and hence its processing along the secretory pathway relies heavily on the co- and posttranslational modifications acquired during the protein transit between the endoplasmic reticulum (ER), Golgi, and trans-Golgi network (TGN) [34] [57]. Like secretion of other salivary proteins and glycoproteins, the trafficking of MMP-9 cargo remains under a strict control of factors that affect the membrane recruitment and activation of various coat and cargo proteins [58] [59] [60].

Of particular significance to the secretory cargo trafficking and sorting through Golgi-trans-Golgi (TGN) is the role of small GTP-binding proteins of the ADP-ribosylation factor (Arf) family [60] [61]. Arfs are small 20 kDa GDP/GTP-binding proteins belonging to the Ras superfamily of GTPases that control protein transport and recycling between different cellular compartments, and cytoskeleton remodeling [60]. The Arfs are active when bound to GTP and inactive when bound to GDP, and the activation status is controlled by the gua-



nine nucleotide exchange factors (GEFs) [61] [62]. All six members of the Arf family are characterized by presence the N-terminal myristoylate tail, which allows their association with membranes, and based on their amino acid sequence homology are grouped into three classes. Of these, class I and class II Arfs are associated with the Golgi apparatus and implicated in the regulation of cell secretory function, while class III Arf, Arf6, functions in the endocytic system [59] [63]. While inactive Arfs are cytosolic, the stimulus activated GTP-bound class I Arfs (Arf1, Arf2, and Arf3) rapidly translocate to the Golgi membrane compartments and assume the principal role in the recruitment of various cytosolic coat and cargo adaptor proteins, exchange factors, and lipid modifying enzymes, including phospholipase D, phosphatidylinositol 4-kinase and protein kinase D (PKD), that are essential for regulation of ER-to-Golgi traffic [58] [60] [63] [64].

### 3.2. Protein Kinase D

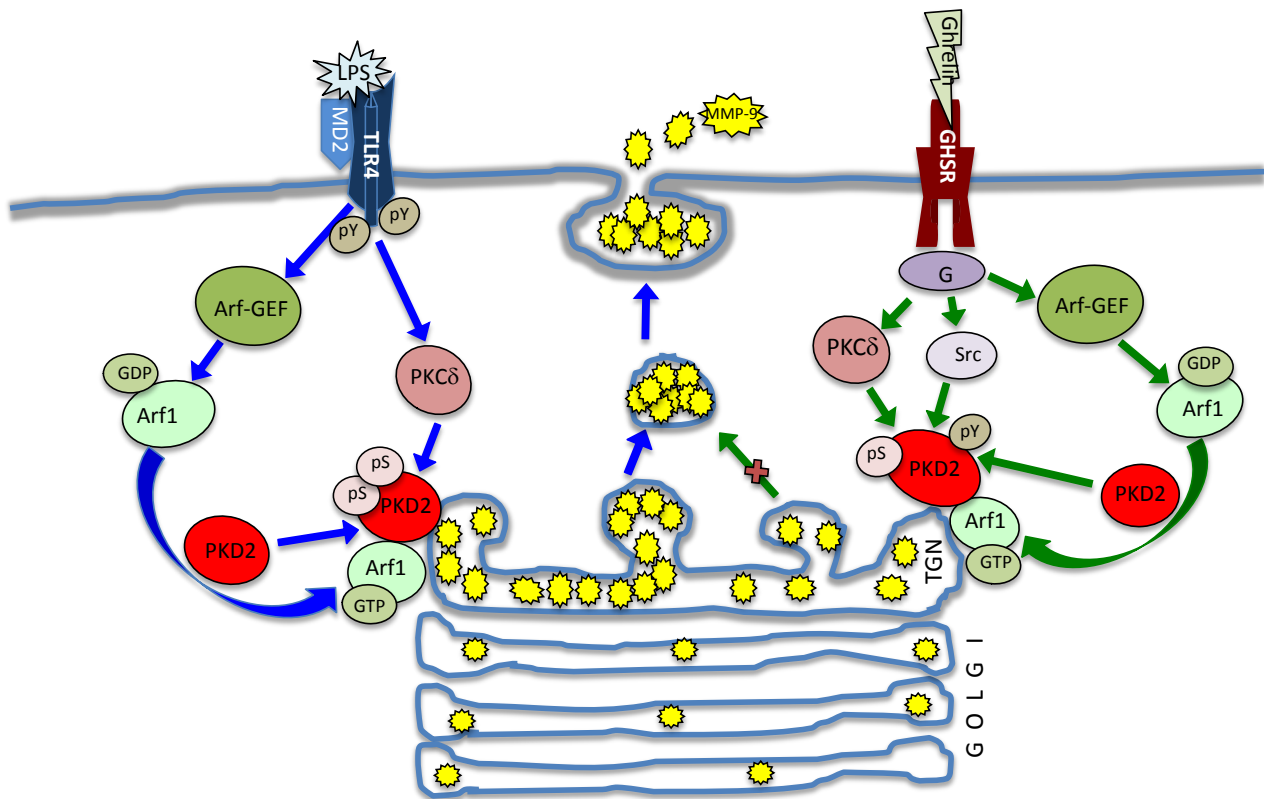
PKD is a family of serine/threonine kinases consisting of three closely related calmodulin-dependent members (PKD1, PKD2, and PKD3), that play a crucial role in the regulation of Golgi structure and function by phosphorylation of the TGN-localized substrates required for subsequent shedding of cargo-containing vesicles [58] [65]. Upon stimulation, the members of PKD family are first recruited from the cytosol to the diacylglycerol-rich membrane environment, including the Golgi complex, via interaction with Arf-GTP and then undergo activation by PKC-mediated transphosphorylation of their activation loop on Ser<sup>744</sup>, followed by autophosphorylation on Ser<sup>748</sup> [58] [66]. Therefore, PKD along with PKC form a key convergence and integration node for signals triggered by Toll-like receptor (TLR) activation as well as those arising through G protein-coupled receptor (GPCR) stimulation.

Thus, it is becoming apparent that PKD/PKC axis is not only the signaling target of LPS-induced TLR4 activation that defines the extent of inflammatory response, but also plays a major role in modulation of inflammation by a peptide hormone, ghrelin [67] [68] [69]. This endogenous ligand for the growth hormone secretagogue receptor type 1a (GHS-R1a), initially isolated from the stomach, and later identified in saliva and the acinar cells of salivary glands, has emerged recently as a principal modulator of the local inflammatory responses to bacterial infection, including that to *P. gingivalis* and *H. pylori* [25] [70] [71] [72]. Remarkably, the anti-inflammatory effect of ghrelin observed during progressive heart failure, associated with periodontal disease, has been linked to the suppression in MMP-9 expression [3] [4] [73]. Moreover, we revealed that *P. gingivalis* LPS-elicited induction in MMP-9 secretion and the modulatory influence of ghrelin occur at the level of MMP-9 processing between the endoplasmic reticulum (ER) and Golgi [74], and is associated with GEF-mediated Arf1 activation and the TGN recruitment of PKD2, followed by its activation by phosphorylation on Ser by the PKC $\delta$ . Whereas the modulatory effect of ghrelin is manifested by the SFK-PTKs-dependent phosphorylation of PKD2 on Tyr

(Figure 3). Hence, Arf1/PKD2 junction could represent a novel target for development of more effective treatments of periodontal disease.

#### 4. Changes in Microtubule Dynamics and MMP-9

Investigations into the processing of MMP-9 along the ER, Golgi and TGN indicate that, in addition to factors that affect the membrane recruitment and activation of various coat and payload proteins, the trafficking of MMP-9 cargo occurs in concert with the changes in stability dynamics of the major cytoskeleton polymeric structures, microtubules (MTs) [56] [75] [76] [77]. Indeed, the literature data provide an ample evidence that cytoskeleton plays an important role not only in cell division and motility, but also is involved in the intracellular transport and positioning of organelles and vesicles, and the MTs have been directly implicated in the regulation of MMP-9 secretion in several different cell systems [56] [76] [78] [79]. The MTs polymers, assembled from heterodimers of  $\alpha$ - and  $\beta$ -tubulin proteins in a head-to-tail manner, form a dense network of



**Figure 3.** Schematic representation of the role of Arf1/PKD2 in mediation of salivary gland acinar cell MMP-9 secretion in response to *P. gingivalis* LPS stimulation, and the modulatory influence of ghrelin. Engagement of TLR4 by the LPS triggers Arf-GEF-mediated Arf activation and its translocation to the Golgi. This promotes the localization of PKD2 with Arf1-GTP at the TGN, and its activation by phosphorylation on Ser by the PKC $\delta$ . The activated PKD2, by acting on the TGN-localized substrates, affects the vesicle fission events and thus enhances the secretion of MMP-9. Ligation by ghrelin of GHS-R1a, on the other hand, leads to Arf-GEF-mediated Arf1 activation and PKD2-to-TGN localization, followed by the SFK-PTKs-mediated phosphorylation of PKD2 on Tyr that maintains the PKD2 activity at its normal regulatory level. G heterotrimeric G protein, pS phosphoserine, pY phosphotyrosine.

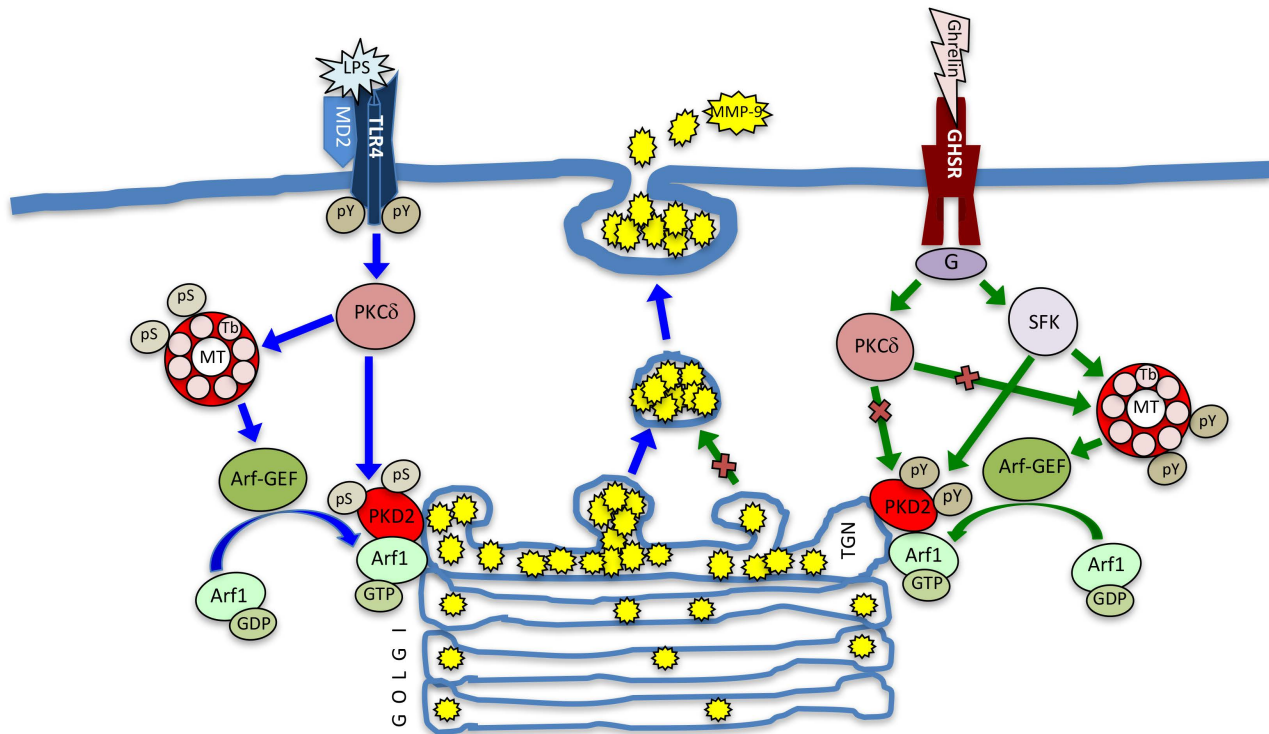


filamentous tubes that undergo frequent alternating periods of growth (polymerization) and shortening (depolymerization) termed “dynamic instability” [77] [78]. Interestingly, the changes in tumor cell stability dynamics are associated with malignant transformation, and the enhancement in MT stabilization along with the increase in MMP-9 production has been observed during inflammation and wound healing [34] [80].

The diverse aspects of MT dynamics arise from accumulation of a variety of posttranslational modifications on the tubulin subunits, including acetylation, deetyrosination, polyglutamylation, polyglycylation and phosphorylation, and are further tuned and refined through binding of MT-associated proteins [78] [81] [82] [83]. The changes in MT stability dynamics and the increase in  $\alpha$ -tubulin acetylation have been directly linked to the increase in MMP-9 secretion elicited by MT stabilizing agents [56], and we have shown recently that MT destabilizing agents that inhibit microtubule polymerization exert the inhibitory effect on the increase in salivary gland acinar cell MMP-9 secretion elicited by *P. gingivalis* LPS [74]. There are also reports pointing to the role of tubulin phosphorylation in MT stabilization, and the evidence suggests the involvement of PKC and Src family tyrosine kinase (SFK) in this process [80] [84] [85]. The role of tubulin phosphorylation in MT stabilization is furthermore, supported the results indicating that phosphorylation of  $\alpha$ -tubulin on Ser prolongs the duration of MT growth, while the phosphorylation on Tyr promotes MT dynamic instability [80] [84] [85].

Therefore, considering that PKC and SFKs play a major role in propagation of oral mucosal inflammatory response to *P. gingivalis* associated with the enhancement in MMP-9 production, as well as are the targets of modulatory influence of a peptide hormone, ghrelin [33] [74] [86], we assessed the influence of *P. gingivalis* LPS and ghrelin on tubulin phosphorylation in salivary gland acinar cells and the MMP-9 secretory processes affected by the changes in MT dynamics [87]. The findings revealed that *P. gingivalis* LPS-elicited induction in the acinar cell MMP-9 secretion is accompanied by the enhancement in MT stabilization, while the modulatory influence of peptide hormone, ghrelin, is associated with MT destabilization. Further, we found that the changes in MT dynamics induced by the LPS and ghrelin occur through signal-regulated  $\alpha$ -tubulin phosphorylation on Ser/Tyr [87]. The LPS-induced TLR4 activation and subsequent up-regulation in MMP-9 secretion was reflected in a marked increase in PKC $\delta$ -mediated  $\alpha$ -tubulin phosphorylation on Ser, while the modulatory influence of ghrelin, an endogenous ligand of the growth hormone secretagogue receptor type 1a (GHS-R1a, a G protein-coupled receptor (GPCR), was manifested in by SFK-PTKs-dependent phosphorylation of  $\alpha$ -tubulin on Tyr (Figure 4). Moreover, the changes in MT dynamics, conferred by the LPS and ghrelin, were found to affect the Golgi localization of GTP-Arf1 and the recruitment and activation of PKD2.

As the elevated levels of MMP-9 are found in gingival tissue of patients with periodontitis, the above findings suggest that factors affecting MT dynamics and



**Figure 4.** Schematic illustration of the role of  $\alpha$ -tubulin phosphorylation and the changes in MT dynamics in modulation of salivary gland acinar cell MMP-9 secretion in response to *P. gingivalis* LPS and ghrelin. Engagement of TLR4 by the LPS triggers PKC $\delta$ -dependent  $\alpha$ -tubulin phosphorylation on Ser and the enhancement in MT stabilization, that leads to the up-regulation in Arf-GEF-mediated translocation of Arf1-GTP to the Golgi, and promotes the recruitment and activation of PKD2. The activated PKD2, by acting on the TGN-localized substrates, affects the vesicle fission events and thus enhances the secretion of MMP-9. Ligation by ghrelin of GHS-R1a, on the other hand, leads to SFK-PTKs-mediated phosphorylation of  $\alpha$ -tubulin on Tyr that maintains MT dynamics at its normal regulatory level. G heterotrimeric G protein, GEF guanine nucleotide exchange factor, MT microtubule, Tb tubulin, pS phosphoserine, pY phosphotyrosine.

MMP-9 secretion present a tempting target for the therapeutic intervention in the treatment of chronic periodontitis. An interesting application of this new type of therapeutic intervention was reported recently the use of spleen tyrosine kinase (Syk) inhibitors to affect MT stability in order to enhance ovarian cell susceptibility to paclitaxel [80].

## 5. Conclusions

MMP-9 is a highly glycosylated zinc-dependent endopeptidase implicated in a wide range of inflammatory and neoplastic diseases, including oral cancer, Sjogren's syndrome, rheumatoid arthritis, and chronic periodontitis, a persistent oral mucosal inflammation attributed primarily to infection by periodontopathic oral pathogen, *P. gingivalis* [5] [6] [7]. Moreover, the elevated levels of MMP-9 in serum of periodontal disease patients have been directly linked to the increase in risk of autoimmune and cardiovascular diseases [5] [9] [35] [88] [89]. Hence, revealing the insights of the signaling cascades involved in the regulation of MMP-9 expression could provide new and innovative options for the treatment of chronic periodontitis and systemic diseases caused by oral infection with *P.*

*gingivalis*.

Based on the presented literature data, it is evident that the induction by *P. gingivalis* in oral mucosal release and activation of MMP-9 relays primarily on its key endotoxin, LPS, engagement of TLR4 and the ensuing activation of MAPK signaling cascades. Indeed, the LPS-induced and TLR4-mediated up-regulation in ERK and p38 activation has been demonstrated to trigger the pathway of GEF-regulated Rac-1-GTP membrane translocation that promotes membrane localization of p38, while ERK was shown to be involved in cPLA<sub>2</sub> phosphorylation, its recruitment to the Rac1/p38 complex, and up-regulation in the activation. Apparently, the activated cPLA<sub>2</sub> by acting on membrane phospholipids affects the membrane fusion events that result in the induction in MMP-9 release. The role of Rac1/p38 complex in cPLA<sub>2</sub> activation is not yet totally clear. There are, however, indications that the complex may be required for the enhancement in cPLA<sub>2</sub> activation by p38-mediated phosphorylation of the phospholipase on the additional Ser sites.

Moreover, it has been established that *P. gingivalis* LPS-elicited induction in MMP-9 secretion, as well as the modulatory influence of peptide hormone, ghrelin, occurs at the level of MMP-9 processing between ER and Golgi, and requires the involvement of factors that control secretory cargo processing, including Arf1 and PKD2. Interestingly, the recent findings indicate that the LPS-induced upregulation in MMP-9 secretion occurs in concert with the changes in stability dynamics of the major cytoskeleton polymeric structures, MTs, which are known to influence the secretory cargo processing and sorting by affecting the Golgi localization of Arf1 and the recruitment and activation of PKD2.

While LPS-induced changes in MT stability dynamics are known to arise through a range of posttranslational modifications of the tubulin subunit of MT, including acetylation and phosphorylation, the *P. gingivalis* LPS-elicited up-regulation in MT stability and MMP-9 secretion has been clearly linked to the increase in  $\alpha$ -tubulin acetylation, while MT destabilizing pharmacological agents were shown to exert the inhibitory effect on the LPS-induced MMP-9 secretion. Interestingly, the modulatory influence of ghrelin on *P. gingivalis* LPS-elicited increase in MMP-9 is associated with MT destabilization. Further dissection of the changes in MT stability dynamics associated with tubulin phosphorylation indicates that the up-regulation in MMP-9 secretion by the LPS is reflected in PKC $\delta$ -mediated  $\alpha$ -tubulin phosphorylation on Ser, while the modulatory effect of ghrelin on MMP-9 secretion is manifested by SFK-PTKs-dependent phosphorylation of  $\alpha$ -tubulin on Tyr. Therefore, the development of new pharmacological agents selectively targeting MT stability dynamics as well as the processing of MMP-9 between ER and Golgi could provide additional arsenal of therapeutic agents of value in the treatment of chronic periodontitis.

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