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CYCLOPHILIN A: A PERIODONTAL INTRIGUE

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ABSTRACT

Cyclophilins (CyPs) are ubiquitous, highly conserved proteins in humans. Cyclophilin A (CyPA) has been found to be a potent chemoattractant, which causes the recruitment of inflammatory cells to the local site of inflammation and thereby contributes to the pathogenesis of periodontal diseases. It has been studied that CyPA is secreted under various conditions such as oxidative stress, hypoxia, and certain infections and hence also functions in different signaling pathways by inducing inflammation via chemotaxis. In this review we intend to widen the horizon on our knowledge of cyclophilin A and its periodontal implications which still remain a mystery.

INTRODUCTION

Cyclophilins (CyPs) are ubiquitous, highly conserved proteins in humans. They have an eight-stranded beta-barrel structure with peptidyl prolyl cis-trans isomerase (PPIase) activity.[1] CyPs have a solvent exposed hydrophobic pocket that serves as the binding site for proline-containing agents and cyclosporine A,[2–4] which regulates the folding and trafficking of proteins.[1,5,6] Cyclophilin A (CyPA) is a highly abundant cytosolic protein composed up of PPIase functional domain.7 It exists in prokaryotes and eukaryotes,[8] was first found in the cytoplasm and accounts for 0.1%-0.6% of cytosolic proteins.[8,9] CyPA is secreted by cells under conditions of oxidative stress, hypoxia, and infection and functions in different signaling pathways by inducing inflammation via chemotaxis.[10–13]

Biological significance of CypA

CyPA is a potent chemoattractant for the recruitment of inflammatory cells and contributing to the pathogenesis of inflammation-mediated diseases. **(Heine et al. 2011; Bukrinsky**

2015)[14,15] Extracellularly produced cyclophilins interact with classical chemokines, resulting in more efficient leukocyte recruitment. **(Arora et al. 2005)**[16] CypA is originally known as the principal intracellular ligand for the potent immunosuppressive drug, cyclosporine (CsA) and has also been reported that CyPA is permanently produced by infiltrating macrophages and lymphocytes, as well as osteoclasts and osteoblasts in periodontitis **(de Oliveira Nobrega et al. 2016)**[17]

There is sparse literature which relates the pathogenesis of periodontitis to elevated levels of cyclophilin A. Most recently **Zhao et al., 2018** elucidated the correlation between cyclophilin A (CypA) and inflammatory infiltrating cells in human periodontitis.[18] The expression of CypA in periodontitis afflicted gingival tissues was determined and highlighted to be associated with leucocyte attraction and anti-inflammatory therapeutics. Smoking is a risk factor for periodontitis that impairs neutrophil functions and since CypA acts as a leukocyte chemotactic factor, **Eren et al., 2017** observed GCF CypA levels to be lowered in smokers though without any significant correlation with chronic periodontitis.[19]

Another marker for macrophage lineage, (including monocytes, histiocytes, giant cells and osteoclasts) is CD68 cells. **(Meng et al. 2016; Radvar et al. 2017)**[20,21] The levels of CD68 have been positively correlated with the pathogenesis of inflammation-mediated diseases. **Lande et al., 2017** have hypothesized that elevated CyPA increases the number of CD68+ cells migrating into the infected sites which cascades the further induction of CyPA.

Extracellular matrix metalloproteinase inducer (EMMPRIN) is a plasma membrane protein best known for its ability to induce the production of MMPs. CypA is related to the inflammatory infiltration and alveolar bone destruction of periodontitis. CypA–EMMPRIN interaction may exist in these pathological processes. In an experimentally induced periodontitis model **Liu et al., 2010,2013** observed elevated levels of CypA and EMMRPIN expressions in inflamed gingival tissues, thereby resulting in further induction of infiltrating cells to diseased sites. These infiltrating cells (mononuclear cells, and neutrophils) further secrete CypA which may aggravate periodontal inflammatory destruction.[22,23]

CypA (Cyclophilin A) is essential for chondrogenic differentiation and endochondral ossification however its effect on osteoclast activity and bone maintenance are oblivious. **Guo et al., 2016** demonstrated low bone mineral density, reduced osteoblast numbers and increased osteoclast numbers in mice calvaria. Thus CypA dually exerts pro-osteogenic and

anti-osteoclastic effects by either gene silencing or its overexpression.[27] Therefore we can hypothesize that regulation of CypA levels in the periodontium can modulate the alveolar bone resorption or regeneration and serve to be useful in treating severe bone or ridge defects or even help in targeting osteoporosis.

Recent researchs conducted in humans as well as animal models have provided compelling evidences which support the critical function of CyPA in several human diseases.[28]The role of CyPA in cardiovascular diseases, viral infections, neurodegeneration, cancer, rheumatoid arthritis, sepsis, asthma and aging have been widely reviewed, though role of CyPA and its potentiality in periodontal diseases needs to be further elucidated to understand its underlying molecular mechanisms and help develop novel pharmacological therapies.

Invitro studies

Kallen et al., 1991	Reported by nuclear magnetic resonance spectroscopy that the X-ray crystal structure of human recombinant cyclophilin was a complex tetrapeptide, to determine its specific binding site for cyclosporin A. Their results describe the structural basis for rationalizing the immunosuppressive function of the cyclosporin-cyclophilin system to determine its importance in designing improved immunosuppressant drugs.[3]
Steinmann et al., 1991	Possible physiologic role of peptidyl-prolyl cis-trans-isomerase, the folding of procollagen I in suspended chick embryo tendon fibroblasts. Previously it was found that the folding of procollagen I is slowed by CsA. The results show that peptidyl-prolyl cis-transisomerase (and hence cyclophilin) accelerates protein folding in living cells.
Zydowsky et al 1992	Studied six site-directed mutants of human cyclophilin A purified from Escherichia coli to homogeneity. Their biochemistry was assayed for cis-trans peptidyl-prolyl isomerase (PPIase) activity, their ability to bind the immunosuppressive drug cyclosporin A (CsA), as well as protein phosphatase 2B (calcineurin) inhibition in the presence of CsA. The results indicate that CsA is a competitive inhibitor of PPIase activity, which can complex with enzymatically inactive cyclophilins and inhibit the phosphatase activity of calcineurin.
Sherry et al., 1992	Isolated an 18-kDa peptide (designated sp18) from lipopolysaccharide-stimulated macrophages. Purified sp18 had in vivo inflammatory activity and in vitro chemotactic activity for human peripheral blood polymorphonuclear leukocytes and monocytes. The results exhibit proinflammatory activity secreted by macrophages in response to endotoxins and also that this protein may function as a cytokine.
Jin et al., 2000	Demonstrated that cyclophilin A is secreted by vascular smooth muscle cell (VSMCs) in response to oxidative stress and mediates extracellular signal-regulated kinase vascular smooth muscle cell. This was the first study to identify CyPA as a redox-sensitive mediator and suggest an important role for CyPA in the pathogenesis of vascular diseases.

Ansari et al 2002	Demonstrated a functional interaction between Cpr1p, Zpr1p (a gene encoding an essential zinc finger protein), and EF1alpha (a translation factor that binds Zpr1p), they even determined the role of Cpr1p in Zpr1p nuclear export, and as a biological function for Cpr1p PPIase activity.
Suzuki et al., 2006	Defined a novel vascular smooth muscle cell growth (VSMC) vesicular secretory pathway for CyPA that involves actin remodeling and myosin II activation dependent signaling events.
Liu et al., 2010	Aimed to address the possible association of CypA with pathological inflammation and destruction of periodontal tissues, and whether CypA-EMMPRIN interaction exists in periodontitis. Experimental periodontitis was induced by ligation. They concluded that CypA expression, is associated with alveolar bone loss, and inflammatory infiltrations and CypA-EMMPRIN interaction may exist in these pathological processes.
Heine et al., 2011	Investigated whether cyclophilin A, (CypA) has the capacity to function with classical chemokines. Neutrophil migration in response to combinations of CypA and macrophage inflammatory protein (MIP-2) was measured by in vitro chemotaxis assays. Thereby providing evidence of greater and more efficient leukocyte recruitment.

Invivo studies

Animal studies

Arora et al., 2005	Demonstrated the significant contribution of cyclophilins to inflammatory responses in vivo using a mouse model of acute lung injury. Blocking cyclophilin-CD147 interactions by targeting CD147 by using anti-CD147 Ab, reduced tissue neutrophilia by up to 50%. These findings demonstrate the significant contribution of cyclophilins to inflammatory responses.
Xue et al., 2018	EMMPRIN-CypA interactions and CD68+ infiltrating cells were applied using mouse monocyte cell line in vitro. A higher level of EMMPRIN and CypA staining was detected in human periodontitis, compared with healthy gingiva. CypA could induce NF-κB activation in the nucleus of mouse monocytic cells in vitro.[29]
Guo et al., 2016	Isolated peptidyl-prolyl isomerase from mice calvaria, the osteoblasts demonstrate low bone mineral density, reduced osteoblast numbers and increased osteoclast numbers, decreased osteogenic differentiation. CypA dually exerts pro-osteogenic and anti-osteoclastic effects.

Human studies

Seko et al., 2004	Analysed the response of cardiac myocytes subjected to hypoxia/reoxygenation by two-dimensional electrophoresis and mass spectrometry. They identified cyclophilin A (CyPA) as one of the proteins secreted from cardiac myocytes in response to hypoxia/reoxygenation and hypothesize that it may protect them from oxidative stress-induced apoptosis.
Liu et al., 2013	Aimed to address the expression and potential role of cyclophilin A (CypA) in the gingival tissues and peripheral blood from patients with periodontitis. Western blot analyses revealed an increase of CypA expression in inflamed gingival tissues. CypA may be involved in the inflammatory response of periodontal tissues through inducing the

	chemotaxis of neutrophils and the secretion of TNF-α/IL-8.
de Oliveira et al., 2016	Evaluated the immunohistochemical expression of matrix metalloproteinase 7 (MMP-7), extracellular matrix metalloproteinase inducer (EMMPRIN) and cyclophilin A (CypA) in periodontal disease, by examining gingival tissue samples. The results suggest that MMP-7, EMMPRIN and CypA are associated with the pathogenesis and progression of periodontal disease.
Eren et al., 2016	Evaluated the gingival crevicular fluid (GCF) CypA and EMMPRIN levels in patients with chronic periodontitis (CP), generalized aggressive periodontitis (G-AgP) by enzyme-linked immunosorbent assay. Higher levels of GCF CypA in patients with G-AgP demonstrated that CypA is associated with the inflammatory infiltrate and alveolar bone destruction of G-AgP.
Zhao et al., 2018	Elucidated the correlation between cyclophilin A (CypA) and inflammatory infiltrating cells in human periodontitis. Western blotting, immunohistochemistry, and immunofluorescence were performed and CD3+, CD4+, CD22+, and CD68+ cells were observed and the NF-κB pathway was activated in the CypA-positive cells in human periodontitis causing an increased leukocytic attraction.

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