Research Paper Medical Science

Proteomics and Periodontal Diseases

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ABSTRACT

The proteins are the building blocks for both microorganisms and periodontium. Periodontitis is the result of complex interrelationship between infectious agents and host factors. The onset, progression and severity of periodontal disease are mainly mediated by various protein molecules. The study of proteins as biomarkers in periodontal diseases has increased attention during the last few years. The proteins involved in pathogenesis of periodontal disease can be used as biomarkers. The knowledge of various proteins involved in periodontal disease pathogenesis can be used in the diagnosis, prevention and treatment of periodontal diseases.The purpose of this article is to review available literature on proteomics in healthy and diseased periodontal structures.

Keywords:Proteomics, proteins, periodontitis, salivary biomarkers

INTRODUCTION

Proteins are vital parts of living organisms, as they are the main components of the physiological metabolic pathways of cells. The word "proteome" is a blend of "protein" and "genome", and was coined by Marc Wilkins in 19961.The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by anorganism or system. This will vary with time and distinct requirements, or stresses, that a cell or organism undergoes. The term "proteomics" was first coined in 1997(James, 1997)2 to make an analogy with genomics, the study of the genes. In simple terms, proteomics is defined as the study of all proteins including their relative abundance, distribution, posttranslational modifications, functions and interactions with other macromolecules, in a given cell or organism within a given environment and at a specific stage in the cell cycle.

Periodontal tissues comprise multicompartmental groups of interacting cells and matrices that provide continuous support, attachment, proprioception and physical protection for the teeth. The periodontium is also specialized to minimize tissue damage arising from trauma and infection. The high level of tissue complexity generated by the multiple types of interacting cells and extracellular matrices, many of which are embedded in very small and difficultto- study compartments, has slowed research in periodontal physiology and pathology. At present there is no catalog of the total expression complement of matrix and cellular proteins in any of alveolar bone, cementum, gingiva and periodontal ligament. Further advances in our understanding of mechanisms of homeostasis and responses to trauma and infection of periodontal tissues will likely require a more complete catalogue of the repertoire of expressed proteins. Currently used proteomics methods can provide global analyses of expressed proteins in specific mammalian cells and tissues.

Recent progress in tissue isolation, protein separation, quantification, sequence analysis, and structural and interaction proteomics offers great promise for bringing periodontal physiology and pathology into the modern era. Yet remarkably few applications of proteomics to the analysis of periodontal tissues have been reported.(McCulloch 2006)3

PROTEOMICS AND DENTISTRY

The two primary areas which dental proteomics have really shown are salivary diagnostics i.e. oral fluid diagnostics or oral fluid biomarkers and proteomics of bone and enamel structures, especially dental enamel. Human saliva contains proteins that can be informative for disease detection and surveillance of oral health. Comprehensive analysis and identification of the proteomic contents in human whole and ductal saliva is a necessary first step toward the discovery of saliva protein markers for human disease detection in particular for oral cancer and Sjogren's syndrome.(Sreedhar , Prakash , Sapna , Santhosh Kumar 2011).4

PROTEOMICS IN PERIODONTAL LIGAMENT FIBROBLASTS

Characterization of periodontal ligament (PDL) fibroblast proteome is an important tool for understanding PDL physiology and regulation and for identifying disease-related protein markers. PDLfibroblast protein expression has been studied using immunological methods, although this technique is limited to previously identified proteins for which specific antibodies are available. A total of 117 proteins have been identified from PDL fibroblasts which can serve as a reference map for future clinical studies as well as basic research. (Reichenberg, Redlich, Cancemi, Zaks, Pitaru, Fontana, Pucciminafra and Palmon, 2005)5

PROTEOMIC BIOMAKERS

Virulence factors from various oral bacteria either cause degradation of host tissue directly or activate a host response. The latter initiates the release of biological mediators from host cells, and when exaggerated in nature, leads to host tissue destruction.Various bacteria-derived enzymes, such as collagen-degrading enzymes, elastase-like enzymes, trypsin-

like proteases, aminopeptidases and dipeptidylpeptidases, are recognized as important participants in tissue destruction. Host and bacteria-derived enzymes, proteins and other inflammatory mediators appear to hold great promise as salivary biomarkers for the diagnosis of oral diseases. Specific salivary proteomic biomarkers have been identified for three key features, namely the pathogenic process– inflammation, collagen degradation and bone turnover. Recently, by using proteomic approach, a reference proteome map of human whole saliva allowing for the resolution of greater than 200 protein spots in a single two-dimensional polyacrylamide gel was deduced. Fifty-four protein spots, comprised of 26 different proteins, were identified using N-terminal sequencing, mass spectrometry, and/or computer matching with protein database. Ten proteins, whose levels were significantly different when bleeding had occurred in the oral cavity, were discussed. These 10 proteins include α-1-antrypsin, apolipoprotein A-I, cystatin A, SA, SA-III, and SN, enolase I, hemoglobin β-chain, thioredoxinperoxiredoxin B, as well as a prolactin-inducible protein. The proteomic approach identifies candidates from human whole saliva that may prove to be of diagnostic and therapeutic significance.(Kathariya and Pradeep, 2010)6

SALIVARY PROTEOMICS FOR EXISTING PERIODONTAL DISEASES

Variable amounts of blood, serum, serum products, GCF, electrolytes, epithelial and immune cells, microorganisms, bacterial degradation products, lipopolyscaaharides, bronchial products and other foreign substances are present in whole saliva.This makes saliva, the best periodontal diagnostic tool. Periodontal inflammatory mediators and tissue destructive molecules have been detected in the gingival tissues, GCF and saliva of patients affected by periodontitis. Saliva contains biomarkers specific for the unique physiological aspects of periodontitis, and qualitative changes in the composition of these biomarkers could be diagnostic. It contains a wide variety of periodontal proteomic markers from immunoglobulins to bone remodeling proteins.

Porphyromonas gingivalis:

Porphyromonasgingivalis is a periodontal pathogen that resides in a complex multispecies microbial biofilm community known as dental plaque. It is a gram negative anaerobe that populates the sub-gingival crevice of the mouth. It is known to undergo a transition from its commensal status in healthy individuals to a highly invasive intracellular pathogen in human patients suffering from periodontal disease, where it is often the dominant species of pathogenic bacteria which commonly resides along with streptococcus gordoniiand fusobacteriumnucleatum. Whole cell quantitative proteomics, along with mutant construction and analysis were conducted to investigate how P. gingivalis adapts to this three species community. The results have confirmed that some 403 proteins were down regulated and 89 proteins were upregulated. The proteins such as HmuR which is up-regulated can be necessary for community structure. (Kuboniwa, Hendrickson, Xia, Wang, Xie, Hackett, Lamont 2009).7

A study in which whole-cell proteomic analyses were conducted to investigate the changes from an extracellular to intracellular lifestyle for Porphyromonasgingivalis ; found that a total of 385 proteins were over expressed in internalised P. gingivalis relative to controls. In another similar study where the change in P. gingivalis proteome was studied as it adapts to a set of experimental conditions designed to reflect important features of an epithelial cell environment, a change in 479 proteins showed evidence of differential expression after different environmental exposure. Further, a study by same authors have shown the shift in the production of cytotoxic fatty acids by intracellular

P. gingivalis, which suggests that the interior of host cells provides a more energy rich environment compared to the extracellular milieu. A similar study on proteome analysis of

P. gingivalis which was placed in subcutaneous chamber of mice showed that PG1385 protein is involved in the virulence of these bacteria. The results of these studies suggest that adaptation to an epithelial cell environment induces a major shift in the expressed proteome of the organism. (Sreedhar et al. 2011).4

Matrix Metalloproteinases(MMP):

MMP-8 a key enzyme in extracellular collagen matrix degradation, derived predominantly from PMNs during acute stages of periodontal disease also correlated significantly with periodontal activity even after adjusting for the confounders. Consequently, quantification of the level of MMP-8 is a promising candidate for diagnosing and, possibly more importantly, predicting the progression of this episodic periodontal disease. Strong correlations between MMP-8 and traditional periodontal diagnostic methods further support the contention that MMP-8 is not only an indicator of disease severity, but also disease activity. Additionally, higher levels of other MMPs, including MMP-2, MMP-3 and MMP-9, were also reported in the saliva of patients affected by periodontitis.

Immunoglobulin (Ig):

Patients with periodontal disease are shown to have higher salivary concentrations of IgA, IgG and IgM specific to periodontal pathogens compared with healthy patients. Additionally, the levels of these immunoglobulins in saliva are greatly reduced after periodontal treatment. As a consequence, the screening of saliva, especially for IgA, is a useful, noninvasive technique to identify individuals who have the potential to develop periodontal disease or those who are currently responding to a periodontopathogenic infection.

Esterase, Lysozyme, Lactoferrins:

The esterase activity of whole saliva is higher in individuals with periodontal disease than in periodontally healthy subjects. Moreover periodontal treatment reduces its levels. Hence the efficacy of periodontal treatment may be readily monitored by changes in levels of activity of specific enzymes like esterase in whole saliva. Patients with low levels of lysozyme in saliva are more susceptible to plaque accumulation, which is considered a risk factor for periodontal disease.Lactoferrin is strongly up-regulated in mucosal secretions during gingival inflammation and is detected at a high concentration in saliva of patients with periodontal disease compared with healthy patients. Numerous other salivary proteases have also been used as diagnostics biomarkers. Various cytokines like C- reactive protein, pentraxin-3, TNF α, various other interleukins which are involved in its pathogenesis have come handy in diagnosing periodontal diseases.

Numerous proteomic markers, like acid phosphatse, alkaline phosphatase, histatins, cystatins, kallikreins&kininogens, aminopeptidases ,aspartate transaminase, glucosidase, galactosidase and glucuronidase and various bone remodeling proteins (Osteopontin, Osteonectin, Osteocalcin) are well known in periodontal diagnosis.(Kathariya and Pradeep 2010)6

CONCLUSION

The use of proteomics and gene expression will advancethe diagnosis and treatment of various oral pathological conditions.In periodontium, many but not all expressed proteins are tissue-specific and the function of various proteins is modulated by multiple factors, including interactions with other proteins and modifications arising from attached phosphates, sulfates, carbohydrates, and lipids. Current proteomics analyses have the capacity to provide new insights into the repertoire of expressed proteins and some inkling of their interactions, at a more global level than previously considered. An important challenge that needs to be met by research workers in periodontology is to embrace proteomics approaches when appropriate, and start to apply them to critical, unresolved questions such as the biological basis for the heterogeneity in gingival, bone, and cementum cell populations.More reviews incorporating other markers and advances in salivary diagnostics are warranted.

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