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Review Article

Proteomics: Diagnostic future in Periodontics?

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Abstract

Periodontitis is an inflammatory condition resulting from the interaction between the infectious agents and host factors. Various protein molecules play a pivotal role in the initiation, progression, and severity of periodontal diseases. The study of proteins as biomarkers in periodontal diseases has been highlighted during the last few years. In periodontitis, multiple bacteria-derived and host-derived mediators expressed in the saliva and gingival crevicular fluid can be utilized as diagnostic markers for the disease. Another significant development regarding human genes and proteins has been the discovery of potential new drugs for the treatment of periodontal diseases. Therefore the information of the proteins involved in the pathogenesis of periodontal diseases can be utilized for its diagnosis, prevention, and treatment.

Keywords: Proteomics; Biological Markers; Periodontal Diseases; Diagnosis

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INTRODUCTION

Periodontal disease is a chronic immunoinflammatory disorder characterized by loss of tooth-supporting structures by advanced host-microbe interactions. The onset, progression, and severity of periodontal disease are mediated by various protein molecules. The information of various proteins involved in periodontal disease pathogenesis helps in the diagnosis, prevention, and treatment of periodontal diseases[1]. The word "proteome" represents the entire protein pool of an organism encoded by the genome. The term "proteomics" is a mix of "protein" and "genome" [2] was first coined in the year 1997 by James to form an analogy with genomics, the study of the genes [3]. To simplify, proteomics is defined as the study of all proteins present in a particular cell or an organism in a given environment and at a specific stage in the cell cycle [4]. In a broader sense, the term 'proteomics' refers to cataloging proteins of a biological subject and the observing of

reversible post-translational modification of proteins by specific enzymes, i.e. phosphorylation, glycosylation, acylation, phrenylation, sulfurization, and so forth. Proteome analysis of bone and dental structure (enamel, periodontal ligament, and cementum) and oral fluid diagnostics (saliva and GCF) are the primary loci where dental proteomics has shown promising outcomes [4]. Also, proteomics helps in understanding the structure and performance of different proteins as well as protein-protein interactions of an organism.

In general, proteomic approaches are often used

i. For proteome profiling,

ii. For comparative expression analysis of two or multiple protein samples,

iii. For the localization and identification of posttranslational modifications,

iv. For the study of protein-protein interactions[5]

The rationale for use in periodontics:

The diagnosis of the dynamic phase of the disease, identifying a patient at risk for periodontal disease, and focusing on early identification of microbial confront to host is tranquil for clinical investigations[6,7,8]. So there has been increasing interest in exploring protein biomarkers to get optimal, novel, and noninvasive approaches for the above-stated causes

Through the last few years, protein as a biomarker in periodontitis has gained confirmation. The study of the proteome, that is, composition, protein-protein interaction, systemic elucidation of protein, extracellular matrix interaction, and posttranslational modification, is in forefront of oral diagnosis. Proteomics so provides systematic/comprehensive data regarding proteins in various tissues and organs [1] to have an excellent beginning for future advancements within the field of diagnostics.

Types of proteomics:

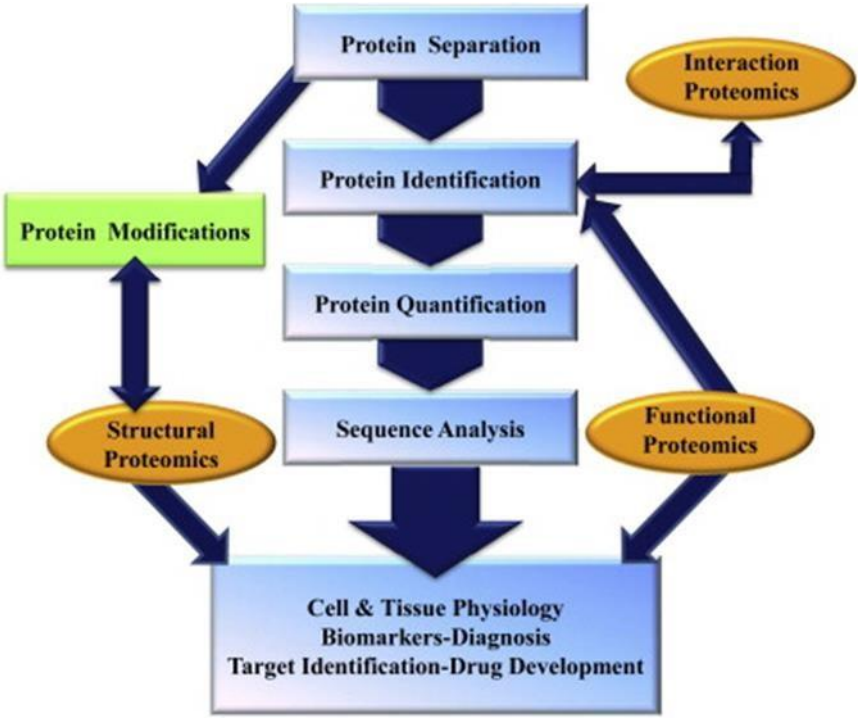
1. Structural proteomics: It is the determination of atomic resolution three-dimensional protein structures on a genome-wide scale in order to better understand the relationship between protein sequence, structure, and function. [9]. The identification of all proteins on a genome-wide scale, deciding their structural-functional relationships, and describing three-dimensional structures are the important hurdles in structural proteomics[10]

2. Interaction proteomics: Within the cell, many processes are governed not only by the relative abundance of proteins but also by rapid and transient regulation of activity, association, and localization of proteins and protein complexes. The association of an unknown protein with partners belonging to a specific protein complex involved in a particular process would then be strongly suggestive of its biological function. The identification of interacting proteins in stable complexes in a cellular system is essentially achieved by affinity-based procedures.[11].The different technique used for this includes yeast two-hybrid system, microassays, and affinity purification.

3. Functional proteomics: Functional proteomics constitutes an emerging research area in the proteomic field focused on two major targets, the elucidation of the biological function of unknown proteins and the definition of cellular mechanisms at the molecular level[12].

Proteome tools:

A flow chart describing the fundamental steps concerned in proteome analysis is given in figure 1



Steps commonly involved in proteomic analysis are:

i. Protein extraction from the sample

ii. Separation of proteins

iii. Peptide fractionation and identification

iv. Storage, manipulation, and comparison of the data using bioinformatics.

Step 1: Protein extraction from the sample

This step involves the extraction of protein samples from whole-cell, tissue, or sub-cellular organelles followed by purification using density gradient centrifugation or chromatographic techniques. Complex samples should be fractionated before analysis to obtain simpler subfractions. The ready sample is then subjected to 2DE (Two-dimensional gel electrophoresis).

Step 2: Protein separation

Two-dimensional gel electrophoresis is applied for the separation of proteins on the basis of their isoelectric points in one dimension and molecular weight on the other. Spots are detected using fluorescent dyes or radioactive probes. It is a powerful separation technique, which permits simultaneous resolution of thousands of proteins.[13]Another technique is Field-Flow Fractionation (FFF) which separates proteins based on their mobility in presence of an applied field, like electrical, gravitational, centrifugal, etc[14]

Step 3: Peptide fractionation and identification

The separated protein spots on the gel are excised and digested in-gel by a protease. The most commonly used enzyme for protein digestion is trypsin. The eluted peptides are identified using mass spectrometry.

Mass Spectrometry: The utilization of mass spectrometry (MS) to spot and characterize biological molecules is a fundamental technology in protein biochemistry and proteomic analysis. The process of protein identification through mass spectrometry is done in two main ways:

i. Peptide Mass Fingerprinting

ii. Tandem Mass Spectrometry

Peptide mass fingerprinting generally uses the masses of peptides derived from a spectrum to examine against a database of predicted peptide masses. These predicted masses are recorded from the digestion of a list of well-documented proteins. Determining amino acid sequence is finally compared with the available database to validate the proteins [15].

Tandem Mass Spectrometry (Tandem MS) utilizes collision-induced dissociation. This method breaks proteins among the peptide backbone and because of this fragmentation, comparisons between the observed fragment sizes and the database of predicted masses is possible comparisons between the observed fragment sizes and the database of predicted masses is possible.

Step 4: Data Analysis

The information obtained from mass spectrometry should be interpreted against protein databases. Sequences are typically determined using one or more search algorithms connected to an appropriate database, such as the International Protein Index (IPI) at the European Bioinformatics Institute or the nr (non-redundant) protein sequence database provided by NCBI. Many software packages are available for the analysis of mass spectrometry data. Commonly used search algorithms include SEQUEST, X! Tandem, and MASCOT [16]

Applications in dentistry and medicine:

Protein detection using highly sensitive proteomic technologies has allowed researchers to accurately monitor changes in biomarker proteins that are representative of the disease. The proteomic analysis examines thousands of proteins at once allowing the detection of specific protein patterns expressed as a consequence of abnormal cellular function or cellular interactions. Analysis of human body fluid proteome has become one of the most promising approaches to the discovery of biomarkers for human diseases. Oral fluid diagnostics, more specifically salivary diagnostics has become one of the widely researched areas in recent years. Protein biomarkers for oral cancer, Sjogren's syndrome, and breast cancer have been recently identified in saliva[17] Also, efforts are being made to apply salivary proteomics for disease-specific biomarker discovery such as lung, gastric, uterine[17,18,19,20,21], and pancreatic cancer[22] Alteration of proteome levels in saliva and GCF with periodontitis has been documented and the identification of these markers could be used as a potential diagnostic tool for periodontal diseases[23]

Proteomics: diagnostic future in periodontics?

Clinical and radiographic assessment of periodontitis remains the basis for patient evaluation. These diagnostic measures for periodontal disease provide information primarily about disease severity and are not useful measures of disease activity. The diagnosis of active phases of periodontal diseases and the identification of patients at risk for active disease represents a challenge for both clinicians and clinical investigators. Under diagnoses within general practice leads to relatively low rates of therapeutic intervention and significant amounts of untreated disease

Based on the information presented, it's clear that the application of proteomics to the medical field has great potential for the improvement of diagnostics and therapeutics. Nevertheless, there are challenges that need to be overcome. It is necessary to have a good understanding of the variability sources that may contribute to error such as pre-analytical, analytical, and biological variation. Pre-analytical variability may be introduced during specimen collection and manipulation, pipetting, and dilution of samples. Careful consideration should be given to specimen collection using different tube types, coagulation times, and storage conditions.

In addition, it is important to account for the biological variability due to gender, age, race, and fluctuations that may occur daily within an individual (biorhythm, fasting, time of the day). All these variables may induce changes that are not pathological in nature but that have to be differentiated from a pathological-induced process.[25]

Choi et al. [24] searched for potential protein biomarkers for periodontitis in a gingival crevicular fluid using LC tandem MS (LC-MS-MS). Azurocidin, an antibiotic protein of azurophil granules with chemo tactic activity, was identified as upregulated in the gingival crevicular fluid. ELISA was then used to verify the upregulation of azurocidin, identifying the latter as a candidate biomarker for inflammatory periodontal disease [25].

Gesell Salazar in 2013 identified characteristic protein patterns that differ in the whole saliva of periodontal diseased and periodontally healthy subjects using a label-free quantitative proteome approach analysis. In total, 20 proteins showed a 1.5-fold difference in abundance between controls and patients ($p < 0.05$); the majority of these proteins showed higher abundance in the periodontally diseased subjects.They concluded that label-free proteomic analysis of the whole saliva is a powerful tool to characterize the periodontal disease status and differentiate between healthy and periodontal disease subjects

CONCLUSION:

Recent advancements in mass spectrometric proteomics provide a promising result in utilizing oral fluids to explore biomarkers for diagnostic purposes. Clinical proteomics offers the promise of biomarker discovery and early detection, diagnosis and prognosis of disease, but major challenges still remain. Further advances in technology are required to eliminate proteomics deficiencies and augment its contributions to the medical and dental field.

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CONFLICT OF INTEREST

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