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PROTEOMICS IN PERIODONTAL DIAGNOSIS AND TREATMENT - A REVIEW

Periodontology

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ABSTRACT

Proteins are essential part of the metabolic pathways of aliving cell and its entire set with the modifications, producedby an organism or system, is considered a proteome. The onset, progression and severity of periodontal disease are mediated by various protein molecules. Periodontal proteomic markers range from salivary protein markers like ImmunoglobulinG to bone remodelling protein markers. Steps involved in Proteomic analysis or protein separation, protein identification, Protein quantification, sequence analysis, bio marker diagnosis. ELISA is a technique for identifying and quantifying proteins. Antibodies against small peptides that are expected to be released by tryptic digestion of cells are specific to the protein under investigation are used in this procedure. ELISA is a technique for identifying and quantifying proteins. Antibodies against small peptides that are expected to be released by tryptic digestion of cells are specific to the protein under investigationare used in this procedure. ProtoMap enables for the detection of post-translational modification proteolysis-induced changes in gel migration. Proteomics and gene expression knowledge will help to improve periodontal disease diagnosis and therapy.

KEYWORDS

Proteome, Genome, Immunoglobulin, ELISA, Matrix Metalloproteinases

INTRODUCTION

Proteins are essential part of the metabolic pathways of aliving cell and its entire set with the modifications, producedby an organism or system, is considered a proteome. The term proteome a blend of protein and genome was coined by Marc Wilkins in 1996.[21]. In simple terms, 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 [24].The onset, progression and severity of periodontal disease are mediated by various protein molecules. Periodontal tissues constitutes multi compartmental groups of interacting cells and matrices that render continuous support, attachment, proprioception and physical protection for teeth.[23]. .The shift from non destructive established gingival inflammation to destructive periodontitis is characterised by alterations in molecular markers.Proteomics is a fascinating research horizon in periodontology that hasthe potential to be a vital tool for the early identification and preventionof periodontal pathologies that are genetically regulated.

Type Of Proteomics

Structural Proteomics

Structural proteomics includes the identification of all the proteins on a genome-wide scale, determining their structure- function relationships, and describing three-dimensional structures of the proteins.The identification of all proteins on a genome wide scale, determining their structural-functional relation-ships, and describing three-dimensional structures are the important hurdles in structural proteomics [30].

Interaction Proteomics

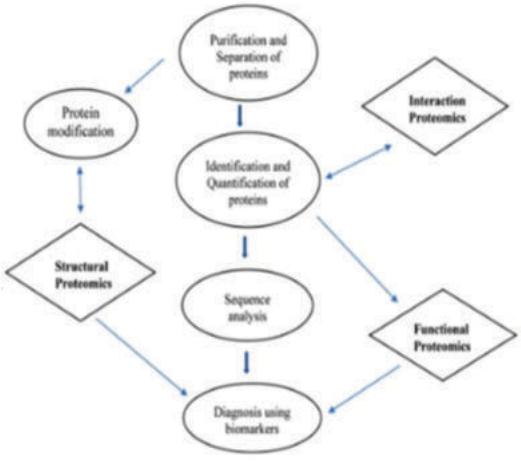
The functions of biological sys- tems are dependent on interactions between their components. These interactions are ultimately determined by genetic elements and selection processes [19]Interactive proteomics focuses at physical intera ctions between proteins and generates disease and pathway-specific protein interaction networks. Proteins' intrinsic chemical and structural diversity, as well as their various expression levels and subcellular localisations, present unique hurdles for network investigation, necessitating the employment of a number of novel and inventive methodologies.

Functional Proteomics

Functional proteomics is a branch of biology that focuses on tracking and analysing the spatial and temporal features of molecular networks and flexus in viable cells [11].It is focused to monitor and analyse the spatial and temporal properties of the molecular networks and fluxes involved inthe living cells . It concentrates on elucidation of biological functions of unknown proteins and cellular activity at molecular level.

Proteins Of The Periodontium

Periodontal proteomic markers range from salivary proteinmarkers like Immunoglobulin G to bone remodeling proteinmarkers [17]. Periodontal proteomic markers range from a wide variety of potential biomarkers like Immunoglobulins (Ig) (IgA, IgG, IgM, sIgA), by-products of tissue breakdown (collagen telopeptides,proteoglycans, osteocalcin, fibronectin fragments and bonecollagen fragments), Pyridinoline cross linked carboxyl terminal telopeptide of type I collagen (pyridinoline, deoxy-pyridinoline,N-telopeptides and C-telopeptide), osteopontin, host factors thatinclude host response cells like monocytes, Polymorphonuclearleukocytes (PMNs), macrophages, IL-1B, TNF-α and PGE2, host cells like immune cells, and periodontal ligament fibroblasts alongwith host derived enzymes like aspartate aminotransferase, acidphosphatase, elastase, cathepsin B, Matrix Metalloproteinases(MMPs) and microbial factors from Treponemadenticola (T.d), Aggregatibacteractinomycetemcomitans (A.a), Porphyromonasgingivalis (P.g), Tanerella forsythia (T.f), ions like calcium, lactoferrin,platelet activating factors and hormones like cortisol [10].



Immunoglobulins (Ig A, Ig G, Ig M, and sIg A) act as an innate defence mechanism of periodontium by interfering with adherence and metabolism of bacteria.Osteocalcin, osteonectin, and collagente-lopeptidases and bone collagen areassociated with local bone metabolism confined to periodontitis and systemic conditions like osteoporosis or metastatic bone cancers Pyridinoline, deoxypyri-dinoline, N-telopeptides, and C-telopeptides are class of degradation molecules which are released systemically during degradation of collagen matrix and bone resorption due to post-translational modification of collagen. They have emerged as valuable proteome markers for bone turnover and arevery specific for periodontal disease [16]. Osteocalcin. It is the most abundant noncollagenousprotein in bone which has specific calcium binding property[20]; it is synthesized mainly by osteoblasts and thus has adominant role in bone remodeling [5].

Osteopontin (OPN). It is noncollagenous calcium binding glycosylated phosphoprotein in bone matrix and isproduced by several cells including osteoblasts, osteoclasts,and macrophages .

Osteopontin level in GCF is significantly correlated with progression of periodontal disease [18].Interleukins and prostaglandins are important inflammatory mediators released by macrophagesand PMNs by the chemoattractant effects of lipopolysaccharide present in bacterial cell wall Protein synthesis in the functioning PDL, data have been obtained studying PDL fibroblast using immunological specific antibodies techniques .Cathepsin B, a cysteine protease whosesource in GCF is mainly from macrophages, contributes toperiodontal tissue destruction by proteolytic activation ofneutrophil procollagenase[31]. The levels of cathepsin B were observed to increase inperiodontitis. Alkaline Phosphatase (ALP) & Acid Phosphatase (ACP) are membrane-bound glycoproteins engaged inpreservation of alveolar bone and renewal of the periodontalligament . Matrix Metallo-proteinases (MMPs) are Host cell-derived enzymes and arean important group of neutral proteinases implicated inthe destructive process of periodontal disease that can bemeasured in GCF .Apart from being a disease severityindicator, MMP-8 also measures disease activity .(MMP-9) degrades collagen intercellularground substance and may serve as a guide in periodontaltreatment monitoring as its level is higher in GCF of thepatients with chronic periodontitis Neutrophils are the first line of hostdefence against periodonto-pathogenic bacteria. Hydrolytic neutral enzymes (elastase, cathepsin G, myeloperoxidase,lysozyme, hydrolases, lactoferrin, and neutrophil collagenaselike MMP-8 and MMP-9) are present in neutrophlic granules[31]. glucuronidase is a lysosomal enzyme that acts as amarker for release of primary granule from PMNs .

Aspartate Aminotransferase Enzyme (AST) is atissue destruction biomarker released from necrotic cellsin GCF and is associated with periodontitis severity [19],Interleukins and prostaglandins are important inflammatory mediators released by macrophagesand PMNs by the chemoattractant effects of lipopolysaccharide present in bacterial cell wall [2]Fibronectin is a glycoprotein which mediates adhesion between cells . Salivary fibronectin is reduced in periodontitis as P. gingivalis fimbriae bind tofibronectin Platelet activating factor [PAF] is a potent phospholipid inflammatory mediator. Significant correlation was found between salivary platelet activating factor (PAF) levels and the extentof periodontal disease .Higher salivary cortisol levels were observed exerting a stronginhibitory effect on the inflammatory process and immune

Steps Involved In Proteomics

Methods Of Proteomic Analysis

Enzyme Linked Immunosorbentassay [Elisa]

ELISA is a technique for identifying and quantifying proteins. Antibodies against small peptides that are expected to be released by trypticdigestion of cells are specific to the protein under investigationare used in this procedure [25].The bound primary antibodiesare detected using a tagged secondary antibody.The signal is influenced by the peptide in solution interfering with the boundpeptide. The signal will be maximum when no free peptide is present,and intermediate when a little level of available peptide is present andminimal when substantial amount of competitive peptide is present[3].ELISA is useful in evaluatingfor periodontitis, reducing periodontal morbidity and increasingperiodontal health.

Mass Spectroscopy

Mass Spectroscopy is a powerful analytical tool for analysing the structure and chemical characteristics of individual molecules, as effectively asquantifying known materials and detecting novel chemicals withina sample [4]A detector that translates the ions intoelectrical signals is used to detect the ions issuing from the lastanalyser and determine their abundance and process the detectorsignals that would be sent to the computer while using feedbackto control the device The most upregulated proteins in chronicperiodontitis compared with controls were BP1, ITGAM, CAP37,PCM1, MMP-9, MZB1, UGTT1, PLG, RAB1B, HSP90B1. Theintegrative data from transcriptomes and proteomics revealed MZB1 as a potent candidate for chronic periodontitis

Protein Topography And Migration Analysis Platform [protomap]

ProtoMap enables for the detection of post-translational modification proteolysis-induced changes in gel migration. The ProtoMap strategy is a degradomics method for screening protease substrates in complicated protein environments [26]To isolate proteins, sodium dodecyl sulphate polyacrylamide gel electrophoresis is performed according to their relative mobility.Using bioinformatics and LC - tandem MS, the extracted protein and peptides are sorted. The data is combined via software to make a peptograph, which is a visual depiction of sodium dodecyl sulphate polyacrylamide gel electrophoresis. [7]In periodontology, it was recently confirmed that regulatory cross-talk occurs across proteases, either by direct cleavage or by targeting the appropriate inhibitors, regardless of protease type or traditional biochemical pathways [8].

Maldi (Matrix Assisted Laser Desorption/ionization)

MALDI aids in the fast identification of proteins in specific combinations [13] Protein identification data was collected via a 4700 proteomic analyser. A neodymium-doped yttrium aluminium garnet (Nd:YAG) laser with a 200 hz repetition rate is deployed to acquire both MS/MS spectra data.The ones with the strongest ion signals are chosen as MS/MS precursors and external calibration with trypsin autolysis peptides [27].

Two Dimensional Polyacrylamide Gel Electrophoresis

It is used to separate complicated protein combinations from biological specimens [15]. The targeted proteins are separated on a 12.5% SDS-polyacrylamide gel electrophoresis after equilibrium upon electrophoretic separation onto 12.5% polyacrylamide gels. Silver staining is employed to monitor the gels and determine proteins [22]

Non Gel Based Proteome Separation Techniques

Multidimensional Liquid Chromatography (MUD-LC) in conjunction with Electrospray Ionisation MS (ESI-MS/ MS) spectra, have acquired popularity as a result of the limitations of traditional proteomic approaches like not being accurate to identify minor spots and techniques like MALDI do not promote the detection of hydrophobic and basic peptides [9]In these methods, complex protein combinations are processed in solution. One or more steps of capillary chromatography are used to fractionate the peptide mixtures which are then analysed by MS in a data-dependant manner.

Methods Of Quantitative Proteomics

SILAC (Stable Isotope Labelling with Amino Acids)
iTRAQTM (Isobaric Tagging For Relative and Absolute Quantification)
Chemical labelling- ICAT (Isotope Coded Affinity Tags)
SELDI-TOF (Surface Enhanced Laser Desorption/Ionisation-Time Of Flight)[14]
Capillary electrophoresis (CE) [12]

CONCLUSION

Proteomics and gene expression knowledge will help to improve periodontal disease diagnosis and therapy. Its utility in dentistry, however, will hinge on how skillfully oral healthcare providers assimilate it in and out of their practices, considering it necessitates a solid grasp of human genetics as well as the adoption of novel diagnostic and therapeutic technology. The use of proteomics and gene expression will advance the diagnosis and treatment of periodontal disease conditions. proteins being an universal component of all biological functions of the body, the scope of proteomics in health and especially the field of periodontology is expected to scale greater

heights. 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.

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