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Proteomics - A Review Article

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Abstract

The study of proteomics mainly emphasis on the structural aspects,functional as well as interactive nature of proteins. The review of this study is mainly to overcome the dental issues in their early stages to provide diagnosis and treatment protocols. Proteomics acts as a tool and it is the study of expression of the protein changes and post translation modifications either in normal or pathological conditions to diagnose and treat their illness.

Keywords: Biomarkers, Bottoms up method, Mass spectrometry, Proteomics, Top down method

Introduction

The word proteomics was first coined by Mark Williams in 1961, which was generally used to describe a mixture of proteins. Proteomics is the study of interaction of proteins in the cells of an organism. Various studies were conducted in both diseased as well as non-diseased individuals at their cellular level to understand the life's processes and to get an idea about the functional, structural as well as the interactive process of the proteins and to analyze how far these proteins would be beneficial for the diagnosis of the diseases. Furthermore 64% of the total human tissues are analyzed to understand the life processes. Totally there are 17,874 proteins where out of which only 1254 proteins have got no function. Identifying human proteome is highly challenging when compared to the human genome.

There are various proteomic tools in-order to analyze the body tissues. Even the proteomic tools have a greater ability to diagnose the diseases at an earlier stage. Some of the examples of proteomic tools where their ability has been proved include Blood, Saliva, Serum, Urine, Cervico vaginal fluid, Gingival Crevicular fluid, Microorganisms, Enamel, Dentin, Pulp, Cementum, Bone, Periodontal ligament, Stem cells, Oral mucosa. The easiest and most effective way to study proteomics is by collecting saliva, it is easily accepted by the individuals. Flesing Y et al (2009) used saliva as a proteomic tool to compare the salivary profiles in Sjogrens syndrome individuals and healthy individuals.

This study emphasized the limitation of the present proteomic knowledge to diagnose and monitor sequel of Sjogrens syndrome development. Wu Y et al (2009) examined the whole non stimulated saliva collected from patients with generalized aggresssive periodontitis. The approach of this study is useful in understanding the etiology of generalized aggressive periodontitis.

Gene present in the DNA do not reflect the cells status and their functional ability. Specific proteins are the one which determine them and are required by the human

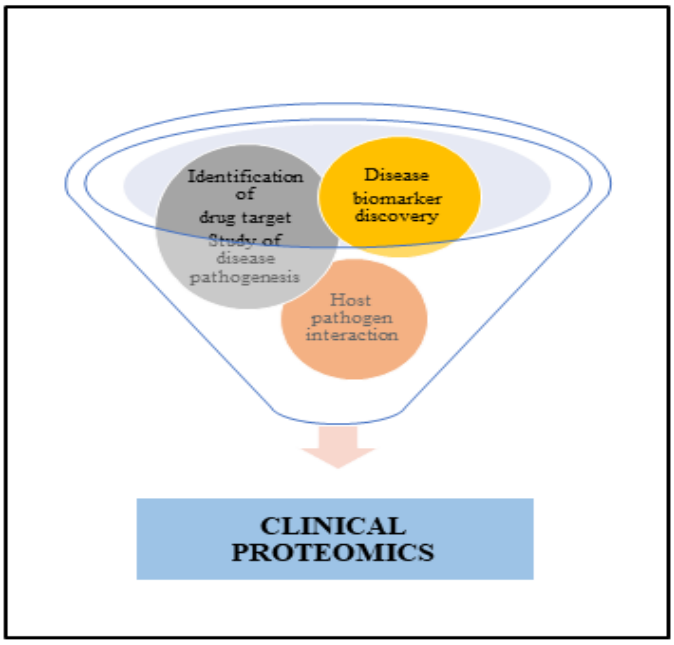
body to perform body functions. Proteins are the cause as well as the cure for various diseases.

Goals of Proteomics

The main goal of this proteomic study includes

1. Protein mapping in healthy as well as diseased individuals
2. Better understanding the functional as well as the interactive nature of proteins.

Methods of Proteomic Study



There are various methods for the study of proteomics. But here we are emphasizing particularly on two methods of proteomic study.

Those include:

1. Top-down method
2. Bottoms up or shot gun proteomics

Proteins are generally produced from amino acids. The combination of essential as well as nonessential amino acids constitute the whole set of amino acids in our human body. All these amino acids are joined by peptide bonds.

Top-down method

In this method proteins are generally analyzed by the peptides where further the analysis is done by Electro spray ionization (ESI) OR by matrix assisted laser desorption generated by gas phase fragmentation method.

Bottoms up method

In this method proteins are generally analyzed by their enzymatic cleavage and their post translational modifications. The other name of this is shot gun method. Grant MM et al (2012) presented a review which focuses on recent advances made in in vivo human periodontal research by use of omic technologies (transcriptomics, genomics, proteomics). The data generated using omic technologies have huge potential to inform paradigm shifts in our understanding of periodontal diseases, analysis and interpretation require a thoughtful and systematic bioinformatics approach, to interpret meaningful conclusions.

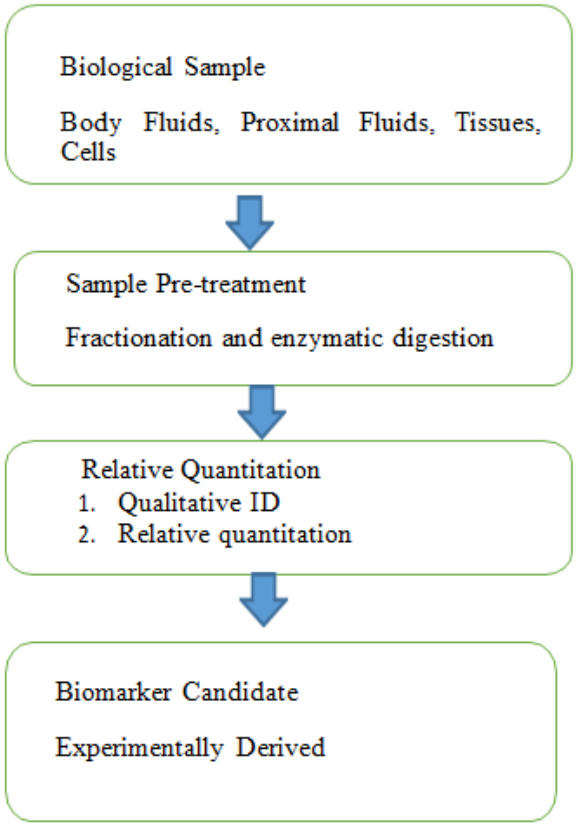
There are various medical tests where the proteomic study has come into practice. Out of all those a few include the following

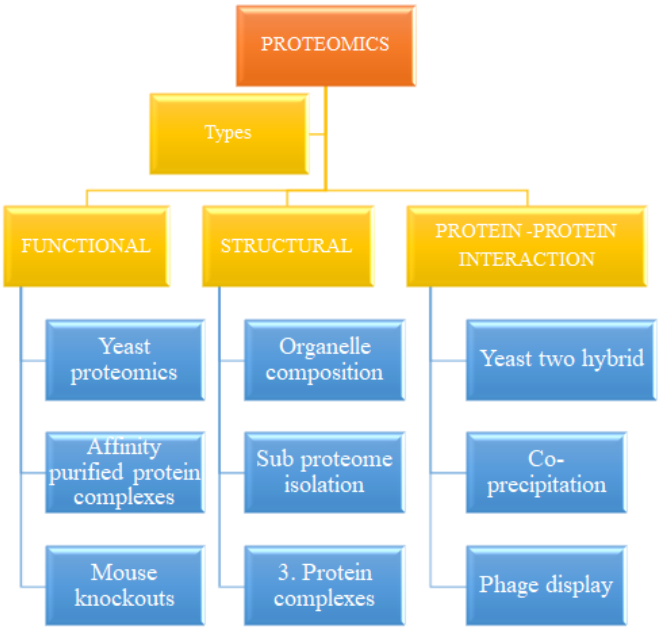
1. Pregnancy test
2. Cholesterol levels
3. Influenza
4. HIV (Human Immunodeficiency Virus)
5. Prostate cancer
6. Pregnancy test
7. Cholesterol levels

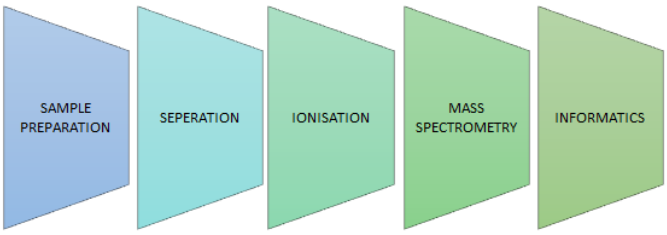
Various drugs have also come into usage where they function particularly over changing the protein structure of the diseases.

Elexacaftor, Texacaftor, Ivacaftor which are used for the treatment of cystic fibrosis to correct their amino acid sequence and restore the normal protein function. Cetuximab (Monoclonal antibody) is used for the treatment of squamous cell carcinoma.

Biomarker discovery and verification







Dental Hard Tissue Proteomics

The dental hard tissues mainly include

1. Enamel
2. Dentin

Enamel has got various proteins which generally determine their secretory as well as maturation phases where they are helpful in determining the structural as well as the functional anomalies in early phases. There are various types of proteins that are present in the enamel which mainly include amelogenin, ameloblastin, enamelin, tuftelin, calbindin. The levels of these proteins in the enamel can be estimated and the anomalies are detected.

Similarly, dentin also has got various proteins whereby determining their levels early diagnosis can be done. These include fibronectin, matrix metallo proteins (MMPS), collagen fibres mainly type 1,3,5,6,12. Jagr M et al (2014) investigated the proteomic profile of human dentin using modern analytical and mass spectrometric techniques. They were able to identify 289 proteins overall, 90 of which had not been previously detected in human dentin. Interestingly, nine 'putative' or 'hypothetical' proteins were identified, mainly classified as immunoglobulins. Those nine proteins were detected, for the first time, as proteins in the human body. Their usage is yet to be detected and helpful in further studies.

Oral Fluids Proteomics

The main oral fluids include

1. Saliva
2. Gingival crevicular fluid (GCF)

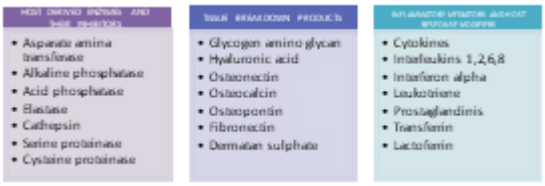
Fleissig Y et al (2010) compared oral fluids (OF) protein expression according to gender and age, using proteomic approaches. There are various changes of protein expression according to age and gender which is helpful for the diagnostic purposes in future.

Saliva

This is the most used diagnostic tool for the study of proteomics. The main properties that is easy accessibility and effective nature of non-invasiveness makes it a major diagnostic tool. Saliva is mainly analyzed by electrophoric and by chromatographic methods. Haigh BJ et al (2010) analyzed the salivary proteome in active periodontitis. This study highlighted the predominant involvement of S100 proteins in the host response during periodontitis, identify host defence components that have not been linked previously to this disease and suggest new potential biomarkers for monitoring disease activity in periodontitis. The various levels of proteins in saliva are analyzed and their levels are estimated followed by their early diagnosis and treatment protocols. Saliva is mainly helpful in diagnosing various oral diseases like oral cancer, Sjogrens syndrome, denture stomatitis. Saliva is mainly collected by RNA pro, SimploFy, Ora gene, Salivette, Saliva bio, Saliva DNA collection device. Range et al (2012) conducted a study to identify salivary profile in obese patients with and without periodontitis. Obese patients have a higher susceptibility rate to periodontal disease due to alpha defensins, which play a major role in gingival inflammation. This study concluded that periodontal status modifies the salivary proteome in obese patients.

GCF

Gingival crevicular fluid plays an important role in diagnosing various gingival and periodontal diseases. Carneiro LG et al (2012) presented a proteomic data on GCF collected from healthy periodontium sites. Baliban RC et al (2012) attempted to identify novel biomarkers collected from GCF samples in chronic periodontitis and healthy individuals. The various biomarkers present in GCF play a vital role for their diagnosis. Measuring the GCF volume measures the biochemical and proteomic inflammation. The various biomarkers of GCF which determine the tissue inflammation include collagenase, elastase, cathepsin B, D, H, L, actin, keratin, histones, annexins, albumin, cystatin B, Ig gamma 1, Ig gamma3, coronin 1A, lactoferroxin. Kido J et al (2012) investigated GCF samples collected from healthy gingival crevice and periodontal pocket by mass spectrometry. This study is useful for understanding periodontal diseases. The various methods for collection of GCF include intra crevicular washings, micropipettes, absorbent paper strips, twisted threads. Out of all these, absorbent paper strip is the easiest way for collection and is also effective.



Soft Tissue Proteomics

1. Pulp
2. Periodontal ligament

Pulp

Pulp comes under the soft tissue proteomics where the biomarkers present in the pulp mainly include annexins hetero ribo nuclear protein C, collagen , matrilin. The levels of these biomarkers determine the defects in the pulpal tissues.

PDL

Periodontal ligament mainly consists of various fibres out of all these mainly type collagen fibres predetermine the condition (both structural as well as functional) analysis. Wu L et al (2009) a study to investigate the differentially expressed proteins involved in the osteogenic differentiation of progenitors presented in periodontal ligament cells. Results suggested that the proteins identified in this study are associated with the unique function of periodontal ligament cells in maintaining periodontal tissue homeostasis, thus providing a comprehensive reference for understanding and investigating details of the molecular mechanisms of periodontal ligament cells involved in periodontal regeneration.

Dental Material Proteomics

Boyan et al emphasized that the protein emdogain has amelogenins which are helpful for the cementogenesis as well as osteogenesis. Dorkhan et al emphasized the effects of saliva or serum coatings which are adherent to streptococcus species to Titanium. Acquired enamel pellicle also is helpful for the proteomic study where they contain certain amino acids like glutamic acid, serine, glycine, proline, alanine, hexosamines. Cementum has got 510 proteins out of which 123proteins -are highly specific to primary dentition 128 proteins -are highly specific to permanent dentition 259 expressed by both. All these are helpful for identification of physiological or the pathologicalevents such as root resorption. Bozic D et al (2012) performed a study on the effect of BMP7 (bone morphogenic protein) on cementoblasts. The bone morphogenic protein is responsible for the differentiation and mineralization of cementoblasts responsible to produce Type1 collagen.

Matrix metallo proteins 8 acts as an important biomarker for detection of early periodontitis and Peri Implantitis. Alveolar bone defects are normally treated by grafts where through the introduction of proteomic study the bone morphogenic proteins (BMP) into the PDL tissue which get differentiated into osteogenic cells which are helpful for alveolar bone formation (non-surgical approach).

Proteomics in Pediatric Diseases

The proteomic study is helpful in the diagnosis of the pediatric diseases. In Mexico a study was conducted on children for the diagnosis of diseases by proteomic analysis. In United Kingdom a study was conducted for understanding the proteomic analysis of cataract in children versus adults.

Cow milk allergy

Pediatric cataract

Cow milk allergy

In recent times individuals particularly in Mexico have stopped breast feeding, so children in Mexico are first exposed to cow milk. In Mexico there are cases, where children started developing allergy related to GIT (Gastrointestinal tract) and Extra GIT associated with vomiting. As of now no symptoms are noted. This study helps in evaluating the exact protein that is present in the milk responsible for such type of allergy.

Pediatric cataract

Pediatric cataract is very dangerous where children lost their lives within a short period (highly life threatening). Crystalline is the major protein where it is responsible for transmission of light to the retina. The levels of this protein in children as well as adults are measured to diagnose the cause, where mostly in UK the cause was due to genetics.

Proteomic Diagnosis in Dentistry

Various protein studies were done for the diagnosis of dental diseases. The levels of various proteins were detected and an early diagnosis was made.

Caries diagnosis

Caries free individuals can be differentiated from caries individuals based on biomarkers present. Caries free individuals have lipocalin, cystatins, proline rich proteins. Caries individuals have Ig A and high levels of amylase. This basis differentiates caries to the caries free individuals where thiscan be a diagnostic measure. Thomas CH (2011) carried out a study to provide a univariate and multivariate analysis of genomic microbial data and salivary mass spectrometry proteomic profiles for dental caries outcomes, characterization of the oral microflora and the salivary proteome associated with health and caries may provide clinically useful biomarkers to better predict future caries experience.

Periodontitis

Chronic periodontitis have Ig G, A, M , lysosome, histatin, CRP(C reactive protein), peroxidase. Gesell SM (2013) used whole saliva samples from periodontally healthy and diseased individuals with chronic periodontitis to screen for disease- associated differences in protein pattern. Aggressive periodontitis has Ig G, A, M, mucins, lactoferrin, histatin, C reactive protein (CRP).

This basis differentiates chronic to the aggressive periodontitis. Lin Z et al (2008) evaluated that PDGF (platelet derived growth factor) play a major role in the control of periodontal regeneration therapy.

Cleft lip and palate: They have actions, cystatins, and keratins as biomarkers for diagnosis and treatment aspects.

Orthodontic treatment: They have Ig alpha 1 chain c region and Ig J chain as biomarkers.

Moebius syndrome: They have high levels of amylase

Leukoplakia: They have high levels of amylase, cystatin, keratin, lysozyme, CK10 (creatininekinase) as biomarkers.

Premalignant lesions: Actin and myosin are major biomarkers for the diagnosis of premalignant lesions.

Proliferative verrucous leukoplakia

Angiotensinogen, peptidyl peptidase act as major biomarkers.

Gingival crevicular fluid (GCF)

- High levels of alkaline phosphatase indicate inflammation and destruction of periodontal ligament.
- High levels of lactate dehydrogenase indicate high levels of probing depths.
- Determining the levels of aspartate transaminase indicate the severity of periodontitis.
- Cathepsin B levels differentiate gingivitis from periodontitis
- Osteocalcin is a major biomarker indicating high bone turnover. Yuan K et al (2007) conducted a study to understand the effectiveness of P 15 (small peptide bone graft). It was concluded that cells treated with P 15 (phosphorous), and an organic bovine mineral were more viable and has more osteogenic activity for the treatment of osseous defects than cells treated with mineral alone.
- Increase in the levels of Osteopontin indicates periodontal disease.

DENTAL ANOMALIES	BIOMARKERS PRESENT FOR THE PROTEOMIC STUDY
Dental caries	Carious tooth has Ig A and high levels of amylase. Caries free individuals have lipocalcin, cyststin and proline rich proteins.
Periodontitis	Increase in levels of Aspartate transaminase indicate periodontitis. High levels of alkaline phosphatase in GCF indicate periodontal destruction. CHRONIC PERIODONTITIS IgG, A, M, lysozyme, histatin, C reactive protein,Peroxidase. AGGRESSIVE PERIODONTITS Ig G, A, M, mucins, lactoferrin, histatin, c reactiveprotein.
Cleft lip and palate	Actin, cystatin, keratin.
Orthodontic treatment	Ig alpha chain c region, Ig J
Moebius syndrome	High levels of amylase
Leukoplakia	High levels of amylase, cystatin, keratin
Premalignant lesions	Actin and myosin
Proliferative verrucous leukoplakia	Angiotensinogen, dipeptidyl peptidase
Bone turnover	High levels of osteocalcin
Gingivitis versus periodontitis	Cathepsin B levels in the GCF Differentiate periodontitis from gingivitis.

Conclusion

Proteomics act as a remarkable tool in the diagnosis, treatment of various pathological conditions in the human body. It is a promising tool to change the practice of dentistry. It has become a challenging aspect for various research workers to start applying these tools and resolve some questions such as biological basis for the heterogenicity in gingiva, bone, cementum.

Further, proteins being the building blocks of human body, their scope of application into the regular practice of dentistry is still limited and expected to scale greater heights. In today's condition and costly lifestyles, it is very much essential to rationalize cost-effective decisions for the diagnosis and treatment of various diseases.

Therefore, through this review we want to endorse SMART (specific, measurable, accessible, and achievable, realistic, time efficient) diagnosis to broaden the aspects for the treatment protocol.

In near future, diseases are completely diagnosed by measuring the level of proteins. In future the damaged teeth particularly by caries as well as by trauma are repaired by stem cell activation and tissue regeneration procedures.

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